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Role of symbiotic relationships in Australian terrestrial orchid conservation
Fungi in agricultural landscapes: implications for eucalypt woodland revegetation
Mycorrhizal fungi of *Prasophyllum*
Small mammals as seed dispersers
And much much more………

SPECIAL THEME: CONSERVING SYMBIOSES

This issue

Regular Features

Australasian Plant Conservation Editorial Team

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Contributing to _Australasian Plant Conservation_

Australasian Plant Conservation is a forum for information exchange for all those involved in plant conservation: please use it to share your work with others. Articles, information snippets, details of new publications or research, and diary dates are welcome. **The deadline for the December 2006-February 2007 issue is Friday 24 November 2006.** The December-February issue will be on the special theme of 'Conserving Grasslands and Grassy Ecosystems'; however general articles are also very welcome. Please contact Tom May if you are intending to submit an article: tom.may@rbg.vic.gov.au.

Authors are encouraged to submit images with articles or information. Please submit images as clear prints, slides, drawings, or in electronic format. Electronic images need to be at least 300 dpi resolution, submitted in at least the size that they are to be published, in tif, jpg or gif format. Guidelines for authors are at: http://www.anpc.asn.au/anpc/pdffiles/APCGuideContrib.pdf.

Please send typed or handwritten articles, no more than two A4 pages (or 1100 words), by fax, mail, email, or on disk. If sending by email, please send as a MS Word (2000 compatible) or rich text format attachment to: tom.may@rbg.vic.gov.au.

Front cover: *Thynninorchis huntianus* and wasp pollinator *Arthrothynnus huntianus* from *Flora of the Otway Plain & Ranges* ... by Enid Mayfield, Linton Press, Geelong, 2006. Pollinator image drawn from photographs by Dr Colin Bower and specimens from Museum Victoria. Used with permission. See review on page 26.
Cover design: Siobhan Duffy.
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From the Editor: introducing 'Conserving Symbioses'

Tom May

Co-ordinator, Editorial Team, Australasian Plant Conservation

Royal Botanic Gardens Melbourne

Partnerships between scientists and land managers or community groups and government are of great importance these days in conservation. Partnerships in nature have been around for millennia, and indeed were probably crucial for significant events in evolution, such as the move of plants onto land.

There is an intricate web of inter-connections between plants and other organisms. These symbioses may benefit both partners (in which case the relationship is called a mutualism), or one partner may benefit but the other not be affected for better or worse, or sometimes one partner benefits and the other incurs some detriment. Organisms involved in symbioses often absolutely rely on the relationship for their survival and reproduction.

The focus of conservation efforts is too often solely on particular plants or animals. The purpose of the 'Conserving Symbioses' theme is to draw attention to symbiotic relationships, because conservation of one organism may depend on an understanding of links with other organisms.

Examples of essential plant symbioses are relationships with pollinators and seed dispersers. Mycorrhizas (literally 'fungus-roots') between plants and fungi are another symbiotic relationship, which is not only out of sight below ground, but also an often overlooked aspect of the biology and ecology of plant and fungus.

Most articles in this issue focus on mycorrhizal relationships between orchids and fungi. The prominence of orchids is not surprising, due to the many rare and endangered species, and their reliance on fungi for germination. Nevertheless, it is worth keeping in mind that most Australian plants (with a few notable exceptions such as in the Proteaceae) are mycorrhizal.

In the opening article, Mark Brundrett provides an overview of the 'Role of symbiotic relationships in Australian terrestrial orchid conservation', covering relationships with both mycorrhizal fungi and insect pollinators. The fascinating connections between orchids and pollinators, such as thynnid wasps, are also mentioned in the review (p. 26) by Katrina Syme of a new book about the flora of the Otway region of Victoria (see cover illustration).

John Dearnaley and Andrew Le Brocque discuss the different kinds of fungi that are involved in orchid mycorrhizas, noting the advances made possible in identification of the fungi by molecular biology techniques. Emily McQualter and co-authors focus on the fungi associated with *Prasophyllum*. A most intriguing finding is that fungi isolated from adult orchid plants are not always effective at germinating seed of the same orchid species.

Magali Wright and co-authors detail the contributions of Royal Botanic Gardens Melbourne to Victorian orchid conservation, highlighting a symbiotic approach which includes hosting of an Australia-wide Cooperative Orchid Conservation website. Presentations at the recent International Conference on Mycorrhiza, held in Granada, Spain, are reviewed by Zoe Smith, who mentions many exciting advances, including the first complete DNA sequencing of a mycorrhizal fungus (a species of *Laccaria*).

Given the undoubted importance of fungi as mycorrhizas in Australian ecosystems, and for iconic Australian plants such as eucalypts and casuarinas, there are remarkably few published scientific studies about mycorrhizas in revegetation. Jacqui Stol and Jim Trappe report on a ground-breaking study which indicates that the mycorrhizal fungi associated with woodland trees disappear from adjacent cleared paddocks. They also assessed the efficacy of different forms of mycorrhizal inoculation on outplanted tubestock, and found that some inoculated fungi failed to form mycorrhizas under the potting mix and watering regime that they used. There is a great interest in using fungi for revegetation (a common question is "where do I buy the fungi") but there is a great deal more to learn before standard protocols can be recommended.

There are numerous other symbioses that do not involve fungi and orchids! The article from Anne Cochrane and co-authors is a reminder of other ways that plants rely on animals. The authors demonstrate that ingestion by small mammals of seeds of *Billardiera fusiformis* enhanced germination; they also discuss the potential for ingestion to aid dispersal.

Articles not on the particular theme of each *Australasian Plant Conservation* are always welcome, and the final two articles feature a report from Kimberlie Rawlings and David Carr on the resurrection of FloraBank, the native seed information and web tool resource, and an

update from Steve Benham of the Auckland Botanic Gardens, describing the Threatened Native Plant Garden, an exciting initiative where threatened plants are showcased in replicated versions of their natural habitats, along with other members of the relevant plant community.

Recent issues of *Australasian Plant Conservation* have been based around a theme, and it is intended to continue with this arrangement. Themes for forthcoming issues include: Conservation of Grasslands and Grassy Ecosystems, Soil Biota in Native Vegetation and Taxonomy and Plant Conservation. Suggestions for themes are welcome, as are articles, especially from parts of the country not well-represented in recent issues, particularly South Australia and Tasmania, and areas of Australasia outside of Australia.

Preparation of *Australasian Plant Conservation* is a team effort and I would like to draw attention to the contribution of the team of volunteers, acknowledged on the inside cover of each issue, who provide information for the Recent Literature and Resources section and who edit and proof-read articles.

Role of symbiotic relationships in Australian terrestrial orchid conservation

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Introduction

Orchid conservation requires a three-dimensional approach to understanding the habitat requirements of (1) the orchid plant, (2) mycorrhizal fungi and (3) insect pollinators. Therefore, specialised knowledge and skills in botany, mycology and entomology are required to work with organisms belonging to three different kingdoms. The incredible complexity of these interactions has amazed and confounded scientists since the time of Darwin (1904) and Bernard (1909), who were amongst the first to study the pollination and mycorrhizas of orchids in detail.

This review focuses on the role of symbiotic associations with mycorrhizal fungi and insect pollinators. Knowledge of orchid demography, genetics, and other habitat requirements is also essential for effective orchid conservation (Fig. 1). Long-term demographic studies determine the viability of orchid populations, estimate rates of transition between seedling, flowering, non-flowering and dormant states and reveal factors, such as grazing and competition, that result in declining populations (see Light and MacConaill 2005). Genetic studies have revealed that the structure of orchid populations is influenced by pollination, seed dispersal, reproductive isolation and hybridisation (Cozzolino and Widmer 2005, Schiestl 2005, Tremblay *et. al.* 2005).

This review focuses on terrestrial orchids of temperate southern Australia and most of the examples provided concern orchids from Western Australia (WA). The information presented here is based on a more comprehensive review, which should be consulted for additional information and literature surveys (Brundrett in press). Readers are also encouraged to consult the proceedings from recent conferences for further information on orchid conservation (e.g. Dixon *et. al.* 2003, Walsh and Higgins 2005).

The problems

Approximately 25,000 species of orchids are known, the majority of which are tropical epiphytes (Chase *et. al.* 2003). Most Australian terrestrial orchids occur in higher rainfall regions of Australia in bioregions where the most threats to biodiversity occur (Commonwealth of Australia 2002, Hopper and Gioia 2004). The orchid family is highly diverse in WA, with over 400 recognised taxa of which 36 are Declared Rare (Endangered or Critically Endangered). Some 39 species are Priority Flora (similar to the IUCN Vulnerable, Data Deficient and Near Threatened categories) and many recently recognised taxa require further evaluation (www.florabase.calm.wa.gov.au). The proportion of rare WA orchids is consistent with other large plant families in WA (Brundrett in press). In contrast, there is a much higher proportion of rare orchids in Victoria (using IUCN criteria), where 65% of 372 taxa are of conservation concern (Backhouse and Cameron 2005). Key threats to rare orchids in both Western Australia and Victoria include habitat loss or modification from disturbance, salinity, weed invasion, grazing by feral and non-feral invertebrate or vertebrate animals, small fragmented populations and drought (Brown *et. al.* 1998, Duncan *et. al.* 2005). Factors that are likely to contribute to the rarity of orchids are summarised in Figure 1.

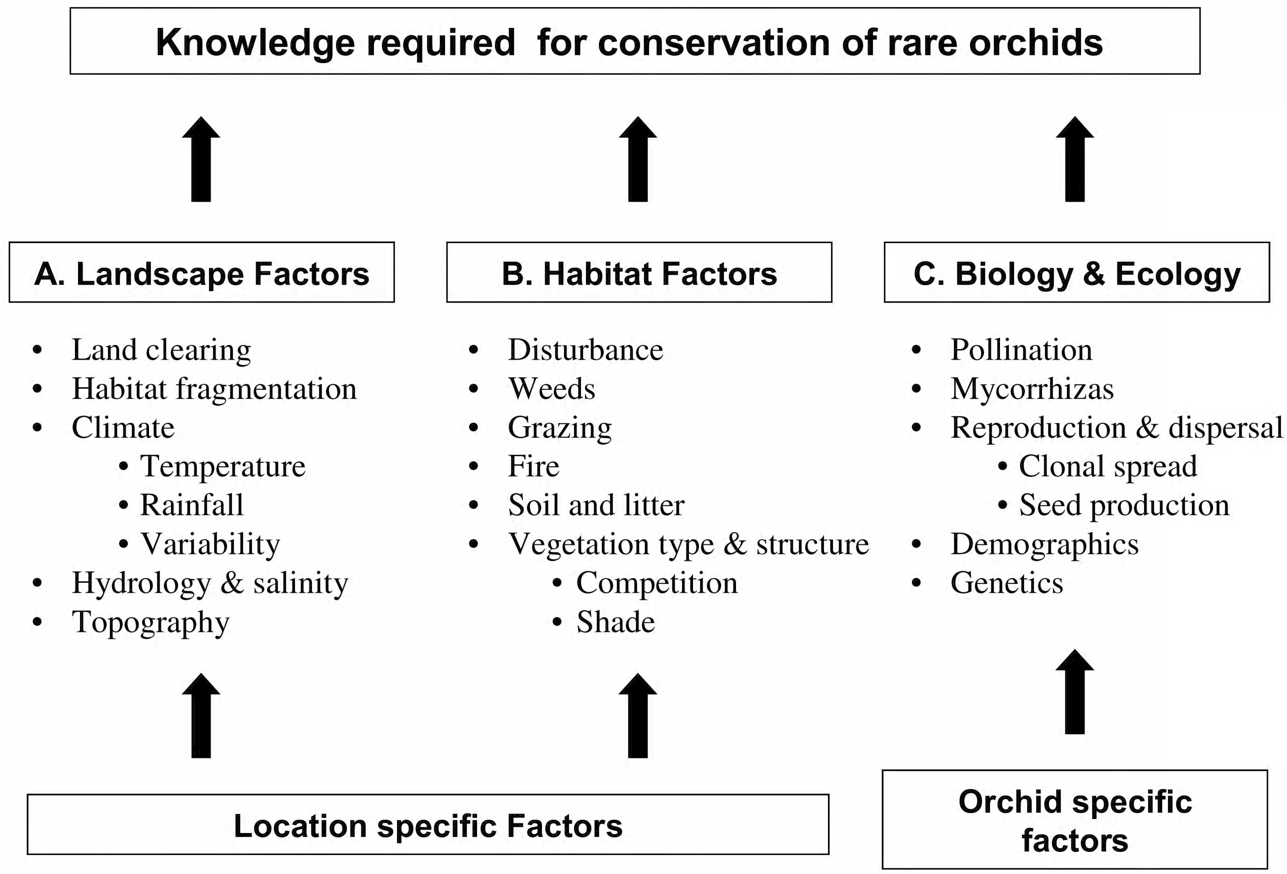


Figure 1. Diagrammatic summary of threats to orchids which operate at (A) landscape and (B) habitat scales. These work in combination with reproductive constraints (C) to limit the abundance and distribution of orchids.

Mycorrhizal fungi

Mycorrhizas are symbiotic associations between specialised soil fungi and plants that are primarily responsible for nutrient transfer (Box 1). Most plants in natural ecosystems have mycorrhizal associations, but little is known about the ecology of their associated fungi (Brundrett 1991). The majority of Australian plants have these associations and the rest have alternative nutritional strategies such as cluster roots (e.g. Brundrett and Abbott 1991).

Box 1. Definition of mycorrhizas, as recently amended to include non-mutualistic associations like those of orchids (Brundrett 2004).

- Mycorrhizas are symbiotic associations essential for one or both partners, between fungi (specialised for life in soils and plants) and roots (or other substrate-contacting organs) of living plants, that are primarily responsible for nutrient transfer. These associations occur in specialised plant organs where intimate contact results from synchronised plant-fungus development.

Orchid mycorrhizas are structurally and functionally unique and consist of coils of fungal hyphae in cortical cells of the root, stem or protocorms (germinating seedlings) of orchids (Rasmussen 1995, Peterson *et. al.* 1998). These symbiotic associations are required to germinate the tiny seeds of orchids and support the nutrition of adult terrestrial orchids (Rasmussen 1995). They differ from the mutualistic mycorrhizas of most green plants, as the fungus can provide energy as well as mineral nutrients to their hosts, without reciprocal benefits from the plant (Rasmussen 1995, Bidartondo *et. al.* 2004, Brundrett 2004, Jolou *et. al.* 2005). Fungi which parasitise plants are well-known, but most people would not be aware that orchids turn the tables on fungi by exploiting them. Mycorrhizal associations are responsible for many of the unique properties of orchids (factors 1-6 in Box 2).

Box 2. How orchids differ from other plants:

1. Dust-like seeds with fungus-dependent germination
2. Partial or full myco-heterotrophy (fungal associations that replace light)
3. Subterranean dormancy for one or more years
4. Growth in/on organic substrates (in many cases)
5. Occur in higher rainfall areas
6. Relatively specific fungal associations
7. Specific and complex pollination mechanisms
8. Disproportionately large and/or complex flowers
9. Many deceive insects to achieve pollination
10. Abundant wind dispersed dust-like seeds

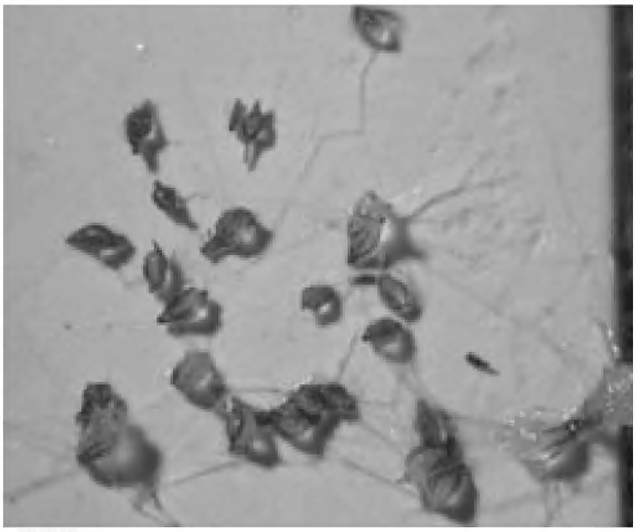


Figure 2. Orchid seedlings germinating over soil organic matter in a baiting method used to detect orchid mycorrhizal fungi in natural habitats (Brundrett et al. *2003).*

DNA-based methods (phylogenetic analysis of sequences) and compatibility testing (germination assays using orchid seed and fungi) have revealed a complex picture of orchid-fungus diversity and specificity (Bonnardeaux *et. al.* in press, Brundrett in press, see also Dearnaley and Le Brocque, p. 7 this issue). While some Australian orchids are known to associate with a range of different fungi, the majority seem to have narrow specificity with a particular fungus or a few fungi. However, even orchids with a relatively high diversity of mycorrhizal fungi have narrow plant-fungus specificity compared to other plants. For example, there is no evidence of plant-fungus specificity for vesicular-arbuscular mycorrhizas, the commonest mycorrhizal association of Australian plants (Brundrett and Abbott 1991).

Because non-mycorrhizal fungi can often be found as endophytes in orchid tissues (Bidartondo *et. al.* 2004, Bayman and Otero 2006, Brundrett 2006) the presence of fungi in orchids alone is not evidence that they are mycorrhizal. It has been recommended that orchid mycorrhizal fungi should be defined as fungi capable of germinating seed of the plant from which they were isolated to an advanced germination stage under controlled conditions (Batty *et. al.* 2002).

The main consequence of fungal specificity is that the occurrence of compatible symbiotic fungi may constrain the distribution of orchids. The presence of compatible fungi in soils is tested by orchid seed bait bioassays where seeds are buried in packets or incubated over organic matter concentrated from soil (Fig. 2) (Rasmussen and Whigham 1993, Brundrett *et. al.* 2003). These bioassays have shown that mycorrhizal fungi are concentrated in coarse organic matter that may be depleted in some habitats (e.g. by tree decline or frequent fire).

Pollination

Complex relationships with insect pollinators are also responsible for some of the unique properties of orchids (factors 7-10 in Box 2). WA orchids have four main categories of pollination strategies (i) nectar-producing flowers with diverse pollinators, (ii) non-rewarding flowers that mimic other plants, (iii) attraction of fungus-feeding insects and (iv) sexual deception (Brundrett in press). The majority of WA orchids have highly specific pollination where sexual deception attracts specific male wasps (Stoutamire 1983, Brundrett in press). Sexually deceptive orchids have flower shapes that help deceive insects. These including Dragon, Flying Duck and Hammer Orchids with a hinged labellum shaped to mimic female wasps, which are some of the world's most amazing orchid flowers (Fig. 3). Sexually deceptive orchids primarily attract insects by pheromones – volatile chemical sexual attractants. Entomologists can identify these chemicals by measuring electrical signals from insect antennae in combination with chemical analysis (Schiestl 2005). These experiments have revealed that *Chiloglottis* and *Cryptostylis* flowers emit the same pheromones as female thynnid wasps (Mant *et. al.* 2005, Schiestl 2005).

The distribution of pollinators also helps to determine the distribution patterns and reproductive success of sexually deceptive orchids, such as Hammer Orchids (*Drakaea* spp), which must flower in the same habitats as the female thynnid wasps they mimic (Peakall 1990). Orchids with a single pollinator are much more likely to have pollination-limited reproduction, than orchids with more diverse pollinators. We know very little about the ecology and distribution patterns of insects that pollinate orchids, but these can be investigated by bioassays (Box 3).

*Figure 3. Sequence of four photos showing thynnid wasp pollinating the Warty Hammer Orchid (*Drakaea livida*).*
Photos: by Bert & Babs Wells/CALM

Box 3. Pollinator Bioassays
This approach was pioneered in WA by Andrew Brown and Warren Stoutamire (e.g. Stoutamire 1983, Bower 1996). Orchid flowers are transported to different locations as insect baits and the presence of pollinators is then noted. These bioassays have revealed new orchid species (Bower 1996, Mant *et al.* 2005).

High rates of diversification (and consequently rarity) are linked to pollination strategies and make it difficult to classify and identify orchids in species complexes within large genera such as *Caladenia* and *Chiloglottis*. Rapid diversification in these genera is driven by strong selective pressure for floral diversity and relatively high rates of gene flow between taxa, both of which are typical of deceptive pollination systems (Cozzolino and Widmer 2005, Schiestl 2005, Tremblay *et. al.* 2005).

Rescuing endangered orchids

Threats to orchid populations listed in the introduction must be addressed by conservation actions in the field. The abundance of rare orchids in Australia has resulted in major gaps in the knowledge and the capacity required to design and implement recovery plans for threatened species. Consequently, effective collaborations between universities, government agencies and community groups are required to manage rare orchids (Marshall *et. al.* 2005). Effective recovery actions must be based on adaptive management principles and adequate knowledge of the biology and ecology of threatened species, summarised as a list of key questions in Table 1.

The first course of action will always be to monitor existing populations and address major threats to orchids (as depicted in Fig. 1) when this is feasible. Indirect recovery actions may not be sufficient to adequately reduce the level of threat of extinction. More direct intervention for the conservation of Critically Endangered orchids will require one or more of the following direct actions; (i) supplemental pollination to increase seed set, (ii) artificial dispersal of seeds into potential habitats, (iii) translocation of adult plants from other habitats, or (iv) outplanting artificially propagated plants or seedlings. Monitoring outcomes is essential to determine the effectiveness of these methods and to measure reductions in threats of extinction. Specific examples of recovery plans that identify threats and describe methods for habitat management, propagation and translocation of Australian terrestrial orchids are available elsewhere (Box 4).

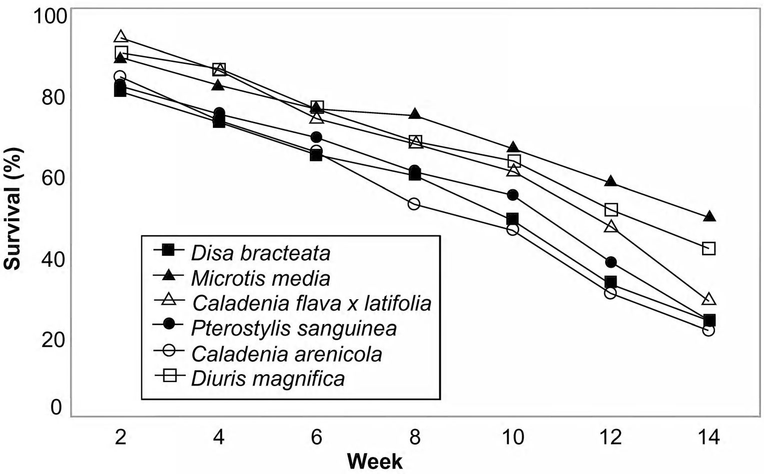
Box 4. Examples of websites where recovery plans and further information on rare orchids are available.

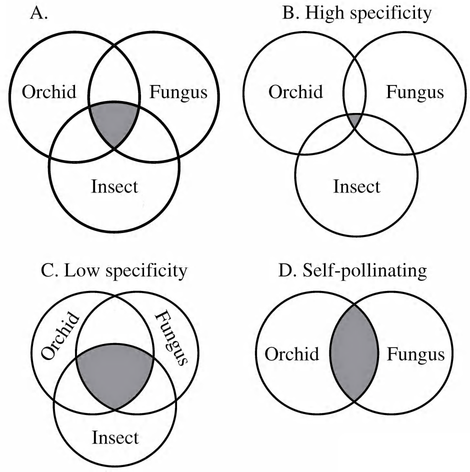
- WA Department of Environment and Conservation (www.dec.wa.gov.au)
- Victorian Cooperative Orchid Conservation website (www.rbg.vic.gov.au/coc)

Epiphytic orchids are commercially propagated in asymbiotic culture using complex agar media, but the propagation of terrestrial orchids has been shown to be most effective by symbiotic germination with a mycorrhizal fungus (Stewart and Zettler 2002, Ramsay and Dixon 2003, Batty *et. al.* 2006a,b). When rare orchids are to be propagated, flowers are usually hand pollinated to ensure seed set, then seeds are cleaned, dried and kept at 4°C for short-term storage or in liquid nitrogen for long-term storage (Batty *et. al.* 2001). Advances in orchid conservation science have resulted in larger seedlings which are more likely to survive translocation (Batty *et. al.* 2006b). Survival of outplanted rare orchids has been demonstrated for several Australian species (Ramsay and Dixon 2003, Marshall *et. al.* 2005, Batty *et. al.* 2006b).

Table 1. Key questions that need to be answered to conserve orchids.

Question	Obtaining answers
1. How many locations and individuals remain?	Surveys of suitable habitats
2. Are populations increasing, stable or declining?	Long term demographic studies
3. What are the main factors influencing the demographics, recruitment and attrition of adult orchids?	Detailed analysis of data from surveys and observations. Supplemental trials of watering, fencing, insect control, etc.
4. How do numbers of flowering plants vary seasonally in response to climate?	Long term demographic studies and climate data
5. What is the frequency of natural pollination and is seed availability a major limiting factor?	Observations of pollination and seed production (pollinator bioassays, supplemental pollination trials).
6. Is natural dispersal to suitable habitats likely?	Spatial analysis of habitat availability, fragmentation and separation
7. Are compatible fungi present in potential new habitats?	Bioassay experiments using orchid seed as baits to detect fungi
8. Are compatible pollinators present in potential new habitats?	Bioassay experiments using orchid flowers as baits to detect insects
9. Are genetic factors suspected to be important?	Test for inbreeding outcrossing rates, etc.
10. What are the survival rates and causes of mortality for translocated orchid seedlings?	Translocation trials to observe survival rates





Left: Figure 4. Attrition rates for seedlings of six species of orchids in urban bushland during the first year (Scade et al. *2006). Seedlings were propagated in the laboratory by a symbiotic germination method (Batty* et al. *2006a).*
Right: Figure 5. A: orchid habitats are defined by three-way intersections between plant, fungus and insect habitat requirements. B, C and D: the relative size and shape of the habitat area where all three of these requirements are met is largely determined by the specificity of interactions between orchids, insects and fungi.

Before orchids can be translocated, potential new habitat must be identified by confirming the following: (i) the physical environment is suitable (see Fig. 1), (ii) compatible mycorrhizal fungi are present, (iii) pollinators are present and (iv) threats listed in Figure 1 are effectively managed. In addition to bioassays for fungi and pollinators, orchid seedlings can be used as bioassays for habitat suitability by transplanting them into the field to identify causes of attrition (Fig. 4).

As shown in Figure 5, there are three separate dimensions to orchid habitat matching: mycorrhizal fungus, insect pollinator and plant. Consequently, studies of the taxonomy, distribution, ecology and biology of symbiotic fungi and insects are important research objectives for rare orchids. We would expect the size and distribution of compatible habitats to vary considerably as a result of the specificity of orchid-fungus, orchid-insect and orchid-habitat interactions (Fig. 5B-D). Orchids with broad habitat requirements include the "weed-like" orchids *Microtis media* and *Monadenia bracteata* which rapidly invade disturbed habitats (Collins *et. al.* 2005). These orchids are not dependant on specific insects (self-pollinating) and have are relatively broad diversity of mycorrhizal fungi (Bonnardeaux *et. al.* in press). Available evidence suggests the majority of rare orchids have both insect and fungal associations which are highly specific, that would contribute to the narrowly defined habitats of these orchids (Brundrett in press).

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Endophytic fungi associated with Australian orchids

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Fungal endophytes of orchids

Australia has a rich orchid flora, with over 1000 native species currently recorded. A significant proportion of Australia's terrestrial orchids are critically endangered, endangered or threatened. Threats to many orchid species include habitat destruction, degradation and fragmentation from increased urbanisation, overgrazing, altered fire regimes and unfortunately, excessive collecting by orchid fanciers. Conservation efforts for Australian orchids include both *ex situ* and *in situ* approaches. *Ex situ* efforts involve the growth of orchid species under horticultural conditions and long term storage of plant and associated fungal material in laboratories and herbaria. *In situ* approaches include re-establishing plants in the wild and protection of current populations through management initiatives.

All orchids depend on fungi for their nutritional needs. As the seeds of orchids are minute and contain very few stored reserves, fungal colonisation is essential for further growth and development following germination (Smith and Read 1997). During plant colonisation fungal hyphae penetrate cells and form elaborate coiled structures known as pelotons (see Fig. 1). A peloton is the site of nutrient exchange between plant and fungus and it is via these structures that young orchids receive sugars and inorganic substances (e.g. phosphorus and nitrogen) necessary for further growth. Because the fungi grow within plant cells they are called endophytes. Mature photosynthetic orchids remain colonised by fungi and supply their endophytes with sugars but continue to receive inorganic nutrients. A number of orchid species (such as *Dipodium* spp), the so-called myco-heterotrophic orchids, completely lack

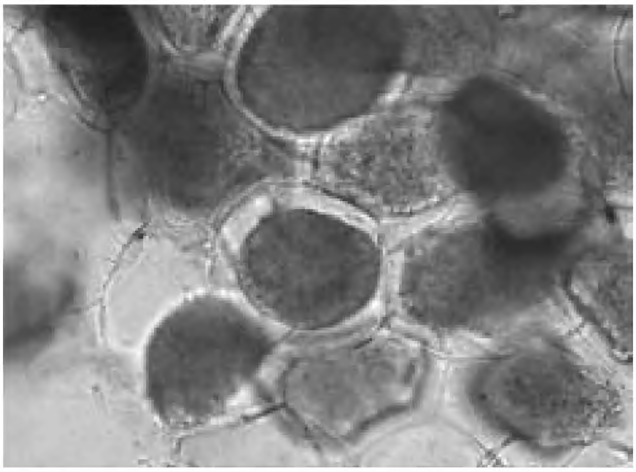

Figure 1. Fungal pelotons inside the root of Dipodium hamiltonianum. *Photo: John Dearnaley.*

photosynthetic capacity and are heavily dependent on a fungal partner to provide both sugars and inorganic nutrients throughout their lifetime.

Fungal endophytes have now been investigated in a large number of orchid species from around the world. The traditional approach to identify the fungal endophytes of orchids has been to isolate pelotons from orchid tissues and to maintain fungal colonies in pure culture. The fungi were then identified on the basis of anatomy and morphology including such features as nucleus number, hyphal cross wall structure and spore dimensions (e.g. Perkins *et. al.* 1995).

Worldwide, the fungi involved with orchids are almost all members of the phylum Basidiomycota, however many do not produce sexual spores, and are consequently assigned to the form genus *Rhizoctonia* (Rasmussen 2002). Form genera are used in fungi when the sexual spores that are essential in the classification of fungi are not produced. The form genus *Rhizoctonia* produces septate hyphae in culture, but there are few other morphological characters to distinguish different species. The presence of *Rhizoctonia* in orchids is intriguing as fungi in this group are usually renowned as serious pathogens of many agriculturally important plant species.

In recent years, analysis of myco-heterotrophic orchid species have shown non-*Rhizoctonia* fungi can also colonise orchids. These are mostly higher basidiomycete genera such as *Thelephora*, *Russula* and *Coprinus*. In addition, various species of *Rhizoctonia* have been matched up to their sexual stages, which occur in genera such as *Thanatephorus* and *Ceratobasidium*. The sexual stage has been induced by altering the culture conditions, often over long periods of growth and with the addition of soil to the cultures, such as by Warcup (1985). Recently, DNA sequence data has also been used to connect *Rhizoctonia* cultures to sexual stages (Bougoure *et. al.* 2005)

Although results may vary with the species of orchid examined, it appears that under natural conditions a particular orchid species may associate with only a few or a single fungal species. This specificity for fungal partners has major implications for orchid conservation. Isolation and perpetuation of the fungal endophyte of a rare orchid species is crucial to growing the plant under horticultural conditions, and explains the high failure rates for attempted propagation of fungal-dependent orchids by fanciers. A further implication is that when undertaking long term storage of rare orchid material, the associated fungus should be preserved as well (Batty *et. al.* 2001). *In situ* management efforts should ensure the longevity of fungal populations as well as conservation of orchid populations.

Endophytes in Australian orchids

The fungal endophytes of Australian orchids have been the subject of numerous investigations over the past four decades (e.g. Warcup 1971, 1981, Perkins *et. al.* 1995, Bougoure *et. al.* 2005). In particular, Warcup (1981, 1990) documented the fungal partners of a large number of mostly common terrestrial orchid species (Table 1). In addition, Warcup (1985) was the first to isolate and identify the *Thanatephorus* fungal endophyte of the rare subterranean orchid *Rhizanthella gardneri*.

Molecular biology techniques identify orchid endophytes

A number of recent studies have been conducted on Australian orchid endophytes using molecular biology techniques. These studies have involved isolation of fungal DNA, sequencing of the ITS (internal transcribed spacer) rDNA region and comparison to fungal ITS sequences in GenBank, which is a worldwide database of DNA sequences. Bougoure *et. al.* (2005) examined the fungal endophytes of six common SE Queensland terrestrial orchids. Three species of the genus *Pterostylis* ('Greenhood orchids') were shown to be colonised only by *Thanatephorus* species,

*Table 1. Fungal endophytes (bold) found in Australian orchid genera by Warcup**

Ceratobasidium	***Sebacina***	***Tulasnella***
Calanthe	*Acianthus*	*Acianthus*
Prasophyllum	*Caladenia*	*Arthrochilus*
Pterostylis	*Cyrtostylis*	*Caladenia*
Sarcochilus #	*Elythranthera*	*Caleana*
	Eriochilus	*Calochilus*
	Glossodia	*Chiloglottis*
	Leporella	*Corybas*
	Microtis	*Cryptostylis*
		Cymbidium
		Dendrobium
		Dipodium
		Diuris
		Drakaea
		Eriochilus
		Lyperanthus
		Microtis
		Orthoceras
		Thelymitra

* sources Warcup and Talbot (1980), Warcup (1971, 1981, 1988, 1990)
\# plus other epiphytic genera listed in Warcup (1981)

while two species of *Acianthus* ('Mosquito orchids') were colonised only by a *Tulasnella* species. *Caladenia carnea* ('Pink fingers') was shown to be colonised only by a *Sebacina* species. Bougoure and Dearnaley (2005) and Dearnaley and Le Brocque (2006) have also shown that the myco-heterotrophic orchids *Dipodium variegatum* and *Dipodium hamiltonianum* (Fig. 2) are specifically colonised by fungi from the family Russulaceae, a group of fungi that are well known as ectomycorrhizal partners on *Eucalyptus* roots. Recently Dearnaley (in press) has shown that the vine-like myco-heterotrophic orchid *Erythrorchis cassythoides* is colonized by a number of fungal species including *Russula*, *Sebacina*, *Coltricia* and *Gymnopus*.

Molecular biology techniques have much to contribute to orchid conservation in Australia. The techniques are objective and highly precise and may reveal the identity of fungal endophytes of orchids that defy identification via traditional morphological/anatomical approaches e.g. non-sporulating fungi. It is essential that the many *Rhizoctonia*-like endophytes that have previously been identified using the traditional morphological approach are more thoroughly characterised via molecular analysis so that potentially serious pathogens are not released into natural situations during *in situ* conservation efforts.

Figure 2. The myco-heterotrophic orchids, Dipodium variegatum *(A) and* Dipodium hamiltonianum *(B) – these plants appears to have a specific relationship with ectomycorrhizal fungi that colonise* Eucalyptus *roots. Photos: John Dearnaley.*

Molecular biology techniques make it possible to view for the first time the populations of previously unculturable fungi that colonise numbers of Australian orchid species, with the promise that identification may provide a guide to appropriate culture methods. At present, the sequences available in GenBank do not fully represent all species of fungi occurring in nature. Therefore the best match to sequences derived from fungi associated with Australian orchids may be to more distantly related fungi. There is a need for more comprehensive inventory of all Australian fungi, so that the full promise of molecular methods in enabling rapid and objective identification of fungi can be realised.

Advances in molecular biology techniques, applied to the study of endophytic fungi-orchid relationships, are poised to contribute significantly to future orchid conservation and cultivation, retaining biodiversity and satisfying the human fascination with this extraordinary plant family.

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Mycorrhizal fungi of *Prasophyllum*

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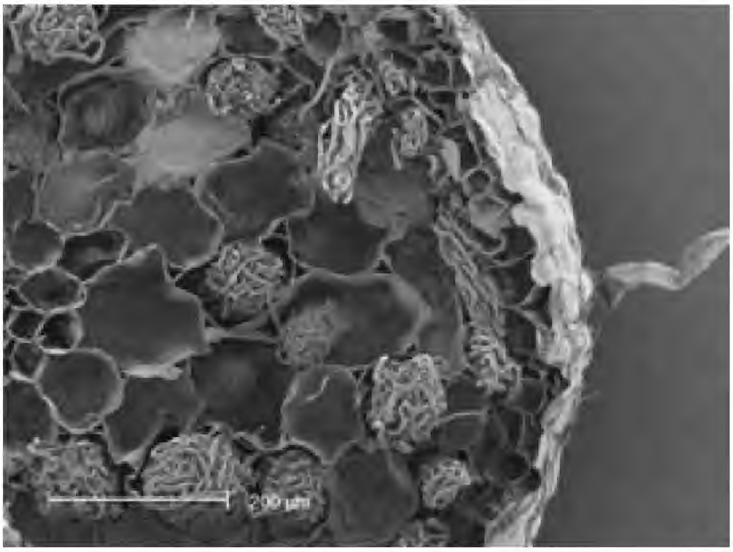
There are more than 380 orchid taxa in Victoria, at least half of which are threatened. The potential extinction of many of these orchids is largely due to habitat destruction caused by degradation from agriculture, industrial development and urbanisation. Effective conservation ultimately depends on reintroduction to field sites so as to reinforce depleted populations. For terrestrial orchids, seed germination is the preferred method of propagation as it allows genetic variability to be maintained (Batty *et. al.* 2006).

The Genus *Prasophyllum*

The genus *Prasophyllum* currently consists of approximately 80 recognised species in Australia and four species in New Zealand (Jones, 1998). Within Australia there are two centres of diversity for the genus, south-western Australia with 25 species (23 endemic) and south-eastern Australia with 50 species. Within south-eastern Australia 30 species occur in Victoria. Most are threatened and restricted in distribution. Overall, it is one of the most poorly known native orchid genera (Bishop, 1996).

Prasophyllum species are obligate mycotrophic plants, which rely on fungi for seed germination. The fungi are also thought to provide nutrients to the adult plants. Current conservation protocols for terrestrial orchids in Australia require propagation with symbiotic mycorrhizal fungi. Unfortunately there is a paucity of knowledge regarding the mycosymbiont of *Prasophyllum*, hampering conservation and re-introduction efforts. Anecdotal evidence has shown that often the mycorrhizal

Left: Prasophyllum *sp.* aff. validum. *Right: The endangered orchid* Prasophyllum diversiflorum.
Photos: Department of Sustainability and Environment Victoria.



Pelotons (balls of fungal hyphae) visible in a section of Prasophyllum *root. Photo: Emily McQualter*

fungi isolated from adult plants do not germinate seed collected from the same plant. Seed germination trials conducted by the Victorian Department of Sustainability and Environment have had no success in germinating *Prasophyllum* seeds in a range of species. One possibility for this lack of success is that the fungi may have been collected at the wrong time of year. Therefore before recovery plans can be implemented for *Prasophyllum,* basic biological information is required regarding the nature of the mycorrhizal relationship.

This study focuses on two threatened *Prasophyllum* species: *P.* sp. aff. *validum* and *P. diversiflorum*, both from south-western Victoria. *Prasophyllum* sp. aff. *validum* grows in low open grassy heathlands and *Prasophyllum diversiflorum* (Gorae Leek Orchid) grows along open watercourses and around swamps on heavy black loams.

Area of Fungal Colonisation in *Prasophyllum*

Following the use of Scanning Electron Microscopy (SEM) it has been found that the area of fungal colonisation in both species of *Prasophyllum* during early leaf development occurs in the roots, particularly in the upper sections of the root. The colonisation primarily occurs in the cortical cells, the fungi entering the orchid through the epidermis and forming balls of hyphae known as 'pelotons' inside the plant cells. The areas that fungi colonise in orchids differ between genera. In *Caladenia* (Spider Orchids) pelotons are primarily found in the stem-collar region of the plants, while in *Pterostylis* pelotons are found in the underground stem (Ramsay *et. a*l. 1986). The morphology of the fungi in both species of *Prasophyllum* is similar but the number of cells colonised appears to be unpredictable. According to Warcup (1981), the main fungus associated with *Prasophyllum* is *Ceratobasidum cornigerum,* although others occur less commonly.

Ex situ orchid seed baiting trials are currently being conducted to determine whether the fungus that is required to germinate the seed is located in soil from sites where the orchids occur naturally. After three weeks the orchid seeds have already reached stage two germination (seeds have swollen, rhizoids developed and meristem is forming), indicating that the compatible fungus is present. However, the fungi isolated from the adult plants have, after two months of trialling, not yet germinated seed. These preliminary results suggest that for both species, the fungi that germinates seed is different to that found in the tissue of adult plants.

Still to Come

Mycorrhizal fungi will be isolated from adult plants at three more times throughout the year: during the period of flower bud growth (winter), while flowering (spring) and as the fruit develops (spring) to determine whether fungal colonisation and type of mycorrhizal fungi changes throughout the different growth periods. The ability for the isolated fungi to germinate seed will be tested with seed from the Millennium Seed Bank at Royal Botanic Gardens Melbourne. As most mycorrhizal fungi from Australian terrestrial orchids do not sporulate in culture and therefore cannot be identified by normal taxonomic means, DNA from fungal isolates will be ITS-sequenced and closest GenBank matches will be determined. The information gained in this study will provide the basis for further re-introduction and conservation studies.

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Royal Botanic Gardens Melbourne contributes to Victorian orchid conservation: *ex situ* propagation with mycorrhizal fungi

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Orchids are fascinating plants. Their complex biology excites naturalists, but makes them vulnerable to extinction because they require the presence of other specific organisms to complete their lifecycle. Their seeds depend on infection by mycorrhizal fungi for germination, and many species can be pollinated only by one insect species. Victoria is a global hotspot for orchid biodiversity, with nearly a quarter of Australia's 1250 species occurring in only 3% percent of the land area. Many of Victoria's orchid species are under threat of extinction, and with nearly 40% endemism (Backhouse and Cameron, 2005) this is of national and even global concern. The aim of the *ex situ* propagation program at the Royal Botanic Gardens Melbourne (RBG) is to provide symbiotically grown orchids as a permanent seed orchard and for re-introduction into natural habitats.

Background

Since the early 1990s, RBG has contributed to the conservation of Victoria's endangered orchids through its *ex situ* propagation program. Research and development, undertaken cooperatively with the Victorian Department of Sustainability and Environment (DSE), the Melbourne Zoo, the Australasian Native Orchid Society (ANOS), The University of Melbourne, RMIT University and Parks Victoria, has led to a greater understanding of Victoria's terrestrial orchids and their associated mycorrhizal fungi, and helped in the implementation of recovery plans for orchid species. One of the strengths of the propagation program is the close collaboration between scientific researchers and people managing endangered orchid populations.

The *ex situ* orchid program

The program is currently focused on more than 45 rare orchid species from the genera *Caladenia, Calochilus, Corunastylis, Corybas, Diuris, Paracaleana, Prasophyllum, Pterostylis* and *Thelymitra*. For each of these species we are attempting to isolate effective mycorrhizal fungi and to germinate seed symbiotically. The field collection of seed and mycorrhizal plant tissue is conducted by staff from DSE, Parks Victoria and ANOS, who send the specimens to RBG, where a researcher isolates fungi and sets up seed germination trials. Fungal isolations are done for different provenances of the same orchid species to ensure any relationships the orchid fungi have with other organisms in a habitat are not compromised, as the role of orchid fungi in the ecosystem as a whole is not well understood. A large living collection of orchid mycorrhizal fungi has been assembled over the duration of the program. These fungi are stored under sterile water, and fungal isolates up to 11 years old have been re-cultured and used for germinating seed. Trials are being conducted to set up cryostorage protocols suitable for the facilities available at RBG. Mycorrhizal fungi are also grown on sterile millet seed for inoculating soil when direct-seeding orchids in natural habitats.

Techniques for propagating and cultivating orchids

Single fungal coils, known as pelotons (Fig. 1), are aseptically removed from the cortical cells of the infected orchid roots or stem collars and plated onto an agar medium in a petri dish. Fungal hyphae grow out from the pelotons after about one week. The hyphae are transferred onto fresh agar (subculture) and any bacterial contamination is eliminated. The fungal isolates are then tested for their ability to germinate seed from the orchid species they were isolated from, in a controlled environment with set temperatures and day length.

Once it is established that the fungus effectively germinates seed, larger quantities of seed are germinated to produce

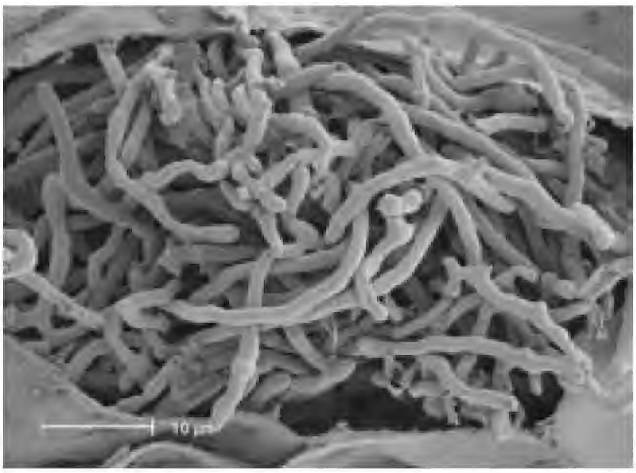

Figure 1. A scanning electron microscope image of a peloton (coil of fungal hyphae) within cortical cells of a Caladenia *seedling. Photo: Magali Wright*

seedlings destined for re-introduction to natural habitats, using a range of fungi isolated from each species and provenance. When the resultant symbiotic seedlings have reached an appropriate size they are transferred to larger flasks where they are able to increase in size more rapidly due to the fresh medium and reduced competition.

Finally, seedlings are transferred from the flasks to tubes of nursery medium in the nursery, and placed into a fog tent with high humidity. This enables them to acclimatise before being moved into a growth house with ambient temperature and humidity. To optimise deflasking techniques, we have trialled different *in vitro* flask designs, nursery potting media, and pot sizes and shapes.

Outcomes

The program has successfully propagated 27 species to date. Once seedlings have become established, the majority will be re-introduced into natural habitats. The re-emergence of seedlings transferred to the nursery in 2005 after the summer dormancy has provided large numbers of some endangered orchid species for re-introduction in 2007. Some species have flowered 18 months after germination including *Caladenia amoena* (Fig. 2), *C. xanthochila* and *C. robinsonii*.

Figure 2. Caladenia amoena *seedlings that were transferred to the nursery in 2005, flowering after re-emergence in 2006. Photo: Magali Wright*

Recognition

The success of the program was recognised with a Banksia Environmental Award in July 2006 in the Land and Biodiversity category. The experience that the program partners have gained and the scientific and management techniques they have developed have relevance to terrestrial orchid conservation in other temperate regions around the globe. Some of this experience will be shared at the International Orchid Conservation Congress in San Jose, Costa Rica in March 2007.

Challenges

For some genera, such as *Prasophyllum, Paracaleana, Corunastylis* and *Calochilus,* locating and culturing an appropriate mycorrhizal fungus has proven difficult. For these genera we are using common orchid species to develop techniques before working on the threatened species. Masters and PhD projects focusing on the specific problems associated with these orchid genera are now underway or being designed.

Further information

Royal Botanic Gardens Melbourne also provides ways for people to learn more about orchid conservation and links interested people Australia-wide through its Cooperative Orchid Conservation website (www.rbg.vic.gov.au/coc/home). A mailing list, called the Australian Network for Orchid Conservation (ANOC), has also been set up to link people interested in orchid conservation and provide a forum for discussion. You can subscribe at http://lists.rbg.vic.gov.au/mailman/listinfo/anoc and join in the conversation about Australia's threatened orchids, and what we can do to understand and preserve them.

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Below-ground thinking: a review of the 5th International Conference on Mycorrhiza, Granada, Spain, 23rd-27th July 2006

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Set amongst the spectacular scenery of Granada, southern Spain, and including a tour of the 'Alhambra', a 13th century Arabian palace and any number of tapas bars, participants of the 5th International Conference on Mycorrhiza were provided with an academic and cultural feast. Entitled "Mycorrhiza for Science and Society", the conference incorporated recent advances in scientific knowledge with their impact on societal demands. The first keynote lecture provided the opening scene: that our heads are in the sand. Although this is literally the case in much mycorrhizal research, in other ways there is much still to learn in understanding the complex multi-trophic interactions above and below ground. ICOM5 brought together people from around the globe, from enthusiastic students to well-published professors, to present a broad spectrum of new and advanced research, ranging from fungal evolution and genetics to the development and maintenance of mycorrhizal interactions and their ecological and economic applications.

Advances in fungal research included a move towards reconstructing phylogenies (evolutionary history) from not just one gene, but from many genes, and the development of new primers for DNA sequencing. Many interesting case studies were presented, such as transplant experiments on a natural post-volcanic desert on Mt Fuji, showing the importance of ectomycorrhizal symbiosis in driving primary tree succession, which may have implications for forestry and agriculture. Other reports dealt with links between functional and genetic diversity in mycorrhizal fungi and preferential allocation of host plant resources to beneficial mycorrhizal fungi indicate the importance of maintaining fungal diversity in ecosystems. Further research is required to identify key host plant species for maintaining mycorrhizal diversity and aiding ecosystem recovery post-disturbance. Shifts in ectomycorrhizal communities were shown to be associated with elevated CO_2 and nutrient availability, however, the numerous environmental variables involved require further research to more fully understand the causes and consequences of changes in atmospheric CO_2 and other pollutants.

New research included the first complete genome sequence of a symbiotic ectomycorrhizal fungus (the mushroom *Laccaria bicolor*), by a team led by the Joint Genome Institute, USA, with the aim of providing critical insights into the genetic makeup of plant-fungus interactions. The complete genome will facilitate characterization of fungal genes involved in heavy metal and salt tolerance mechanisms.

Arbuscular mycorrhizal fungi (AMF) colonisation was shown using biochemical and molecular methods to increase pathogen defense responses and disease resistance in host plants. Tolerant fungi are being used in remediation and restoration of polluted and degraded soils. In addition, AMF were shown to enhance the production of antioxidants in basil and tomato and may have beneficial roles in commercial food production. The isolation and characterization of the first monosaccharide (carbohydrate) transporter in a fungus from the Glomeromycota was reported. Bi-directional transfer of carbon between a green orchid and its fungal symbiont, and a fungus-dependent pathway for organic nitrogen acquisition by an orchid provide the first evidence of mutualism in orchid mycorrhiza. The involvement of fungi from the family Coprinacaceae in orchid mycorrhiza was also revealed for the first time.

Inside the Alhambra, Granada, Spain. Photo: Zoe Smith

Some novel techniques presented were high-throughput TILLING (Targeted Induced Local Lesions in Genome), which is based on screening mutagenised plants for mutations in the selected genes, virus induced gene silencing for comparison of multiple genes simultaneously, and real-time PCR for quantification of extraradical soil mycelium and fluorescent real-time PCR for localising fungal gene expression in symbiotic plant tissues. Other new techniques included fungal parentage analysis using microsatellites and assessment of cryptic species using recombination tests.

Afternoon workshops were energetic, reducing the sleepiness associated with banquet lunches, and allowed for active discussion, particularly when the question of scientific advice for land managers arose. Whilst research has been largely short-term and therefore does not provide long-term answers, can we keep asking questions and wait forever? A recurring observation during the conference was the need to focus future questions towards applied research, and to link science with industry by providing land managers with advice for manipulating mycorrhizal symbioses for restoring ecosystems or in agricultural production. Technical challenges should be addressed by the scientific community as well as industry, not only to prevent gimmick selling of fungal inocula, such as 'foliar sprays', but also to assess the quality of commercial inocula and evaluate their ecological risk, in order to promote sustainable practices in plant production systems. More than 118 posters were presented on biodiversity and ecological impacts of mycorrhiza, indicating a growing area of new research.

A close vote followed two fantastic presentations from India and Brazil, with Brazil taking the mascot to host the ICOM6 in 2008. I would like to commend Chairman José-Miguel Barea and all of the organisers and contributors for an outstanding experience at the 5th International Conference on Mycorrhiza.

Fungi in agricultural landscapes: implications for eucalypt woodland revegetation

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Introduction

Mycorrhizal fungi are a major component of the soil microbiota in many ecosystems. Eucalypts and many other members of the Myrtaceae are highly dependent on mycorrhiza formation for survival and growth. These plants form a particular type of mycorrhiza, the ectomycorrhiza (EM), where fungal hyphae form a sheath around plant rootlets. Mycorrhizas are highly evolved, mutualistic associations between soil fungi and plant roots providing the eucalypts with mineral nutrients and the fungi with photosynthetically derived carbon compounds. Mycorrhizal fungi also assist plants to repel parasitic organisms, obtain limiting soil nutrients, and ameliorate adverse soil conditions and severe climatic conditions by improving water relations. Healthy eucalypt woodland sites generally have abundant propagules of EM fungi, however sites that have been cleared for grazing, eroded, or otherwise degraded may be depleted of these important fungi. Such sites are often where revegetation projects are undertaken in response to significant and widespread problems in agricultural landscapes such as tree dieback, changed water tables or a decline in biodiversity. In such cases, acceptable revegetation or plantation performance can be enhanced by the planting of seedlings with existing good EM formation. Inoculation of tree seedlings with spores has proven so effective in improving plantation performance in the United States that collection of spores has become a significant business (Brundrett *et. al.* 1996 and 2005; Mitchell and South 1992; Steinfeld *et. al.* 2003; Tommerup *et. al.* 2002).

Research questions on fungi, eucalypts, remnants and paddocks

We undertook a one year pilot project (June 2004-2005) to address three main questions:

1. What is the distribution of EM inoculum along a gradient from remnant vegetation into a paddock?
2. Is inoculation of nursery stock with woodland soil and/or spores effective?
3. What is the effect of different inoculation treatments on eucalypt seedling survival, growth and drought response when seedling are planted out in paddocks?

The first question was addressed in an experiment utilising a transect. In a typical heavily cleared paddock used for sheep grazing or cultivation in the Southern Tablelands, soil samples were collected along a 100 m transect from the edge of a Yellow Box (*Eucalyptus melliodora)* / Blakely's

Red Gum (*E. blakelyi*) remnant woodland into the non-treed grassy paddock (Fig. 1).

The soil samples from each station along the transect were blended with potting mix in forestry tubes, then sown with Blakely's Red Gum seed which were to act as 'trap' or 'bait' plants for the bioassay of any EM propagules. The germinated eucalypt seeds were grown for three to four months to allow the mycorrhizal fungi to develop association with the roots. The roots were washed clear of soil and examined under the microscope. Different morphological types of EM were counted and the percentage area of the roots covered by the EM was assessed.

If the presence of EM on root material was difficult to determine, the material was hand sectioned and staining procedures were applied to identify plant root cell types and cell wall components and associated EM fungal hyphae using a compound microscope (Fig. 2).

A second experiment evaluated the effectiveness of different sources of EM inoculation. There were three treatments: samples of eucalypt woodland soils, the spores of a Dyeball fungus (*Pisolithus albus*), and a combination of both soil and spores as EM inoculum. The woodland soil treatment was blended with potting mix, as for the transect experiment, while the *P. albus* spores were applied as slurry to established Blakely's Red Gum tubestock. A non-inoculated control was grown in potting mix without added woodland soil or spores. The control also served to monitor glasshouse contamination from air-borne spores. All tubestock was grown according to Greening Australia (GA) methodologies. GA is one of the main organisations responsible for revegetation projects in these landscapes. Spores were collected from wild populations of *P. albus* and soils were collected from four large eucalypt woodland sites in very good condition. Bioassays were undertaken on eucalypt roots from a sample of seedlings using the method described for the first experiment.

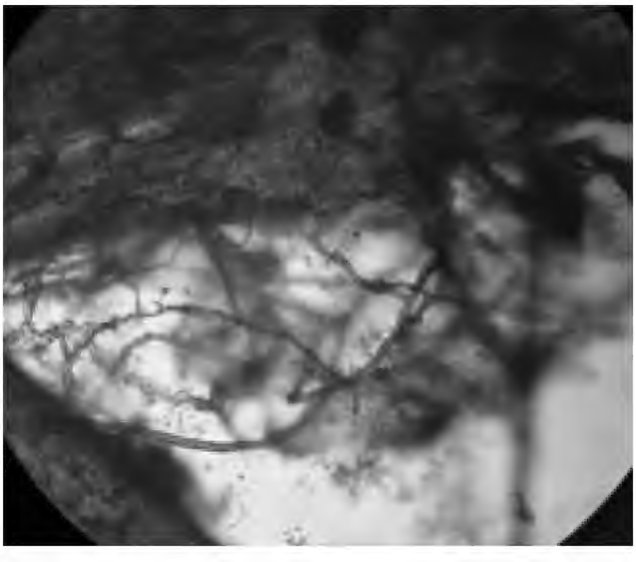

Figure 2. Eucalypt EM root cross section showing plant cell wall structure (above) and thin strands of fungal hyphae (below). Photo: Jacqui Stol

Figure 1. Soil samples were collected along a 100 m transect extending from a Yellow Box / Blakely's Red Gum remnant woodland patch into a grazed or cultivated paddock. Photo: Jacqui Stol

To address the third question, a total of 600 Blakely's Red Gum seedlings from the four treatments in the second experiment were planted into three typical sheep grazing paddocks planned for revegetation (Figs 3, 4, 5). After three months these were assessed for seedling survival, growth and drought response.

Did we find any eucalypt EM fungi?

A total of 32 EM morphotypes were identified from trap plants germinated in soil taken from the woodland-paddock transects and from the inoculated seedlings. The different EM types were separated on the basis of their colour and morphology and ranged from long, brown, pinnate (regularly branched) EMs, caramel brown smooth mantled forms to 'fuzzy' mantled apricot coloured EMs (Fig. 6). The majority of EMs were found in the tubestock inoculated with woodland soil or from trap plants grown in soil collected from the woodland area of the woodland-paddock transect. There was minimal EM formation on control plants from the inoculation experiment.

Do any eucalypt EM fungi remain in sparsely treed paddocks?

The highest proportion of eucalypt rootlets colonised by EM fungi was associated with soil from the woodland edge with an overall reduction of colonisation of root area and species richness with distance from the woodland edge into the cleared paddock. The percentage of rootlets colonized with EM fungi was low apart from among

Figure.3. Tubestock at 4 months old being grown out and hardened off just prior to out- planting as part of the third project objective to assess survival rates between tubestock inoculated with woodland soils, P. albus *spores or a combination of both. Photo: Jacqui Stol*

plants inoculated with woodland soil (less than 2% on average). From the woodland edge into the first 10 m of paddock tubestock averaged 2-3 EM morphotypes and had up to 6% of their root area colonised by EMs. By 20 m this had dropped off to less than one EM morphotype and 1% colonisation.

A total of twenty-two EM morphotypes were detected when soil from the woodland edge was added to tubestock. The diversity of EM morphotypes declined with distance of the soil away from the woodland; rapidly dropping to less than 15 at 10 m into the paddock, 10 at 20 m then staying at less than 5 beyond that. The woodland edge, therefore, appears to provide a source of inoculum for the paddock soil – the closer to the edge, the more abundant and diverse the inoculum.

In summary, soils at the woodland edge and within 10 m of the woodland produced the highest frequency of EM formation on the roots of the *E. blakelyi* tubestock used as host (trap) plants. This interval also produced the highest diversity of EM fungi. Paddock soils sourced at distances of 50 to 100 m from the woodland edge produced negligible EM frequency and diversity on inoculated tubestock, and the EM level did not differ from that found on non-inoculated seedlings.

Comparison of different inoculation treatments

Inoculation of tubestock with woodland soil produced more EMs morphotypes (17) and a higher percentage of EM rootlets colonised than either the addition of *P. albus* spores, the combination of *P. albus* and woodland soils or the non-inoculated controls. In fact no typical *P. albus* EM were evident on any tubestock. The *P. albus* spores not only produced no identifiable *P. albus* EM but also inhibited EM formation when mixed in with woodland soil inoculum. The woodland soils were modestly effective as EM inoculum of tubestock, but the frequency of EM rootlets was unacceptably low (mean of 5% frequency and 2.6 fungal morphotypes) in terms of current nursery forestry practice. In the USA it is generally considered by mycorrhiza researchers that 50% or more of rootlets should form EM if inoculated with appropriate fungi. The drought of the past several years may have depleted the propagules in the soil, and higher rates of soil mixed with the nursery potting mix may be required to boost colonisation.

So far, *P. albus* spores have not been very successful in inoculation attempts by other researchers, although inoculated as cultured mycelium *P. albus* is a very effective EM fungus in peat-vermiculite substrates. It may be inhibited by bark in the potting mix, a standard mix used in Australian nurseries. It is also possible that a typical glasshouse watering regime of three waterings a day is not conducive to EM formation as there is no moisture stress to trigger it. Spores of other fungi, such as species of *Laccaria*, *Hydnangium* and *Descomyces*, have shown good results in other studies (Brundrett *et. al.* 2005) and could be tested for use on tubestock destined for planting in paddocks.

Above: Figures 4 and 5. 600 eucalypt tubestock with the four inoculation treatments being planted out in three paddocks in May 2004 with CSIRO, Greening Australia and a Green Corps team. Photos: Alex Drew and Jacqui Stol

Inoculated tubestock outplanted into paddocks

In August 2005, three months after planting, the 600 seedlings in the three revegetation paddocks were assessed in the field for survival and leaf desiccation. There were some differences among the tubestock in seedling vigor and health, but overall survival was high and there was minimal leaf desiccation. It had been initially expected that any differences in survival and growth due to differences in EM inoculation would be rapidly detectable due to the complete absence of soil moisture at the time of planting with the region well into its fourth year of drought. However one month after planting significant rains filled the soil moisture profile to capacity. This situation highlights the unpredictability associated with field trials of this type. Plantings will be assessed again in Spring 2006. It is expected that if any significant differences between inoculation treatments are detected these would be in the first two years of growth.

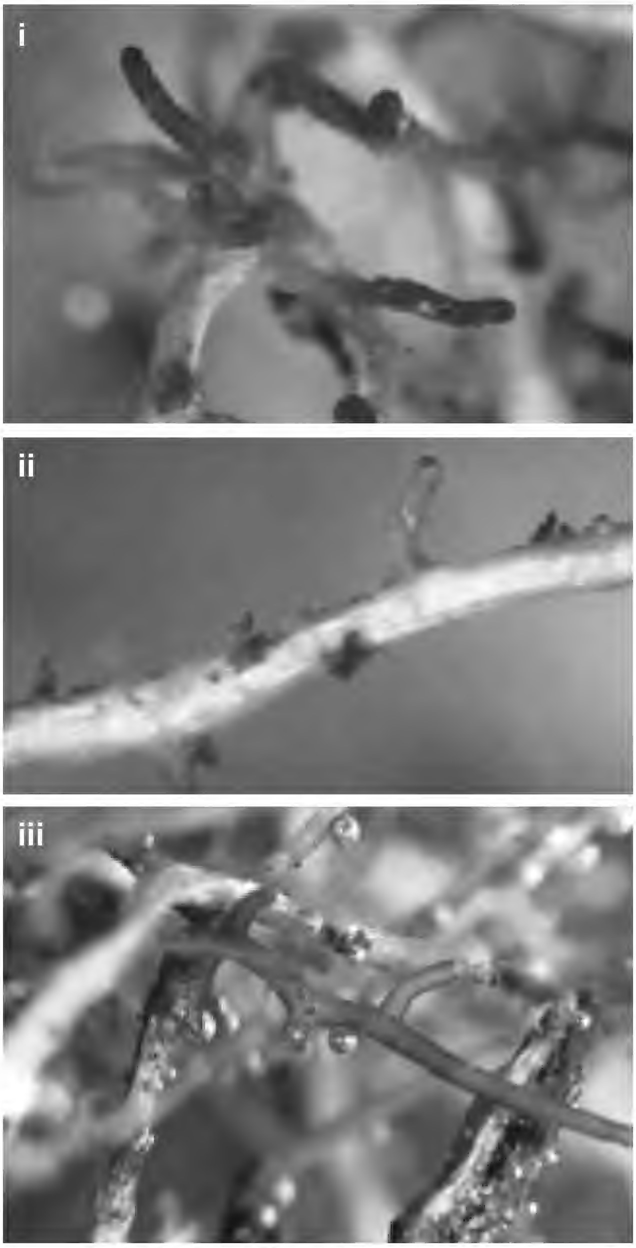

Figure 6. Some of the ectomycorrhizal morphotypes found on Blakely's Red Gum rootlets from paddock transects and inoculations. Photos: Jacqui Stol.
i) morphotype with black tips
ii) bronze morphotype
iii) brown pinnately branched morphotype

Drought effects on inoculated tubestock

An eight week experimental glasshouse treatment to induce water stress on 200 seedlings from the four inoculation treatments showed a similar level of desiccation and there were no differences observed between treatments. Treatment 1 (inoculation with woodland soil) showed gradual leaf death until 75-100% of leaves were desiccated. Treatment 2 (inoculation with *P. albus* spores) showed a similar decline to Treatment 1. Treatment 3 (inoculation with woodland soils and *P. albus* spores) showed a slightly slower decline than Treatment 1 and 2. Treatment 4 (control / no treatment) showed a slower decline than either Treatment 1 and 2 and a slightly slower level of desiccation in comparison to 3. These results, demonstrating little difference between inoculation treatments and a slower rate of desiccation in the control, are believed to be related more to initial glasshouse watering conditions and differences in initial plant condition than to the different inoculation treatments. That is, those plants that were healthy and well developed (most of the 'control' tubestock) showed less desiccation than those smaller plants that had received less water (due to inherent variations of glasshouse watering system).

Conclusions

The results to date of these studies indicate that areas of paddock more than 20 m from a woodland edge are likely to have low EM inoculum potential both in terms of numbers of propagules and EM fungus species richness. Woodland soil can be an effective inoculum, but *P. albus* spores are not, at least under our experimental conditions. Effective inoculation of tubestock may need careful management of glasshouse watering regimes, designed specifically to enhance inoculation. *P. albus* fruits abundantly in association with eucalypts in disturbed soils with low organic matter, such as road verges, suggesting that its spores are effective under such conditions, but it is clearly not a good candidate for spore inoculation or for sites with relatively high soil organic matter. Other fungi, such as *Laccaria*, *Hydnangium* and *Descomyces* species, should be investigated for their effectiveness as inoculant.

Eucalypts are mycorrhiza dependent, so producing seedlings with a strong EM root system can be expected to enhance early establishment and growth in paddock revegetation programs. Given the likely deficiency of EM propagules in paddock soils, EM inoculation of tubestock for paddock revegetation plantings could enhance planting success through 1) the development of strong root systems for tolerating drought, 2) rapid vertical growth to overtop grass and weed competition, and 3) potentially better resistance to insect and mammal damage.

INTRODUCING NATIVE FUNGI INTO REVEGETATION

The experiments discussed above used woodland soil as a source of inoculation for EM fungi, to provide important background information on the mycorrhizal status of soils.

We recommend, however, that when sourcing inoculation **DO NOT COLLECT SOIL** from native vegetation because this can cause irreversible damage to existing native vegetation and also risks transfer of disease causing organisms.

For further information on using fungal spores as a source of inoculation see the FUNGIBANK website (www.fungibank.csiro.au) and the article by Neale Bougher in *Australasian Plant Conservation* 14(1): 3-5 (2005)

Our preliminary study indicates that current nursery methodologies are highly successful in growing plants but are not necessarily conducive to EM formation even if EM inoculum is present. Effective inoculation would require a change to the typical nursery management, by careful control of potting mix ingredients and fertilizing and watering schedules. A protocol for growing seedlings with good EM inoculation is needed that could be adopted by nurseries. A number of private and public nurseries in the United States now routinely inoculate their tubestock.

Acknowledgements

We would like to gratefully acknowledge the significant contribution made by Dr David Freudenberger, CSIRO Sustainable Ecosystems in initiating and supporting the experimental concept and Greening Australia, particularly Brian Cumberland, for coordinating and locating outplanting sites, liaison with landholders and Green Corps, and providing glasshouse facilities and nursery materials. Dr Teresa Lebel at Royal Botanic Gardens Melbourne provided expert guidance on identification of EM types and methodologies.

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Small mammals as seed dispersers

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Animals have the potential to play a key role in vegetation dynamics, assisting in plant succession and maintenance of floral and fungal diversity. Ingested seeds and fungal spores pass through the gut and into the soil resulting in dispersal to new sites. Seed dispersal is important for reproductive success and is the critical mobile stage of a plant's life history. Ingestion and subsequent dispersal of seeds (endozoochory) may increase their germinability by removal of dormancy. Passage through the gut may alter the seed coat or endocarp, aiding water permeability and thereby affecting germination through chemical or mechanical action (Baskin and Baskin 2001). The movement of seeds away from parent plants can provide benefits through colonisation of new areas, escape from seedling mortality near parent plant due to competition between parent and seedlings, and the creation of wider population genetic structure.

Seeds in mammal scats

In this investigation, 275 scats from quokkas (*Setonix brachyurus*), Gilbert's potoroos (*Potorous gilbertii*), bush rats (*Rattus fuscipes*) and quendas (*Isoodon obesulus*) were collected from Two Peoples Bay Nature Reserve, 30 km east of Albany on the southern coastline of Western Australia. Cochrane *et. a*l. (2005) provide a comprehensive presentation of the investigation. In brief, scats were air-dried after collection, weighed and seeds manually extracted. Seed type and load per scat were recorded and the mean number of seeds per scat and per gram of scat calculated for each mammal species. Seeds retrieved from scats were identified by comparison with seeds found on plants in the study area.

Billardiera fusiformis. *Seeds (below) and dried fleshy drupe (above). Photo: Anne Cochrane*

Results

Seeds were found in less than one third of the faecal samples from quokkas, Gilbert's potoroos and bush rats. No seeds were recovered from the quenda scats examined. Scats ranged in weight and number of seeds retrieved (Table 1), with no visible evidence of damage to seeds noted.

Germination of seeds from scats

Seeds of *Billardiera fusiformis* (Labill.) Payer (Pittosporaceae) were germinated as they were the only seeds found in the scats of more than one mammal species. This Australian endemic evergreen climber has blue bell-shaped flowers and cylindrical fleshy drupe containing 30-50 seeds embedded in the mucilaginous pulp. Seeds were incubated without treatment on filter paper over sponge (moistened with 5 ml deionised water) in 90 mm Petri dishes in a 15°C incubator with a 12 hour photoperiod. In addition to germinating seeds from freshly collected faecal matter, seeds recovered from faeces were germinated after more than one year of storage (approx. 15 months in dry storage at 21°C). Where enough seeds were available a smoke treatment was applied to aged seeds.

To compare germinability of ingested seeds with that of non-ingested seeds, freshly collected and aged seeds of *B. fusiformis* were incubated (after flesh removal) under the same conditions.

Fresh non-ingested seeds from plants of *B. fusiformis* were dormant on collection; smoke application increased germination to 12%. Germination of seeds of *B. fusiformis* freshly retrieved from scats was highest from quokka scats (58%) and lowest from bush rat scats (2%), with 31% germination of seeds from Gilbert's potoroo faeces (Table 2). Ageing of ingested and non-ingested seeds increased germination significantly. There was also a significant interaction between ageing and smoke treatment on germination of both freshly collected and ingested seeds of *B. fusiformis.* For these treatments, mean time to germination was more than 30 days for all but the aged or smoke-treated seeds.

Discussion

This investigation has identified a functional relationship between mammals at Two Peoples Bay and the Australian bluebell, *Billardiera fusiformis*. Ingestion assists germination of 'fresh' seeds of *B. fusiformis*. Germination was greater and more rapid in seeds retrieved from scats than in freshly collected seeds. Germination was also greater in seeds collected from plants and aged, indicating an after ripening requirement that implies the presence of primary dormancy. Chemical and mechanical abrasion and de-pulping of seeds apparently combine with the moist faecal environment to stimulate germination and partially overcome this dormancy. Pulp removal from *B. fusiformis* seeds occurred more rapidly through gut passage than by natural decomposition that may take several months. Release from germination inhibitors and high osmotic pressures by removal of flesh are mechanisms that can alter germination rate or percent. For *B. fusiformis* smoke and seed ageing were partner cues for germination in the absence of ingestion to break dormancy.

Differences between dispersers in their effects on seed germination might be explained by difference in their digestive tract morphology and gut retention time. Little of the fleshy fruit was removed during passage of seeds through the gut of bush rats, perhaps contributing to reduced germination amongst seeds in their faeces. The different speeds of germination promoted by different rates of gut passage may increase the probability that seeds

Table 1. Occurrence of Billardiera fusiformis *seeds in scats of four ground-dwelling mammals at Two Peoples Bay Nature Reserve, Western Australia. Reproduced from Cochrane* et. al. *(2005).*

Mammal species	Total no. scats	Mean scat weight (g) n=10	Total scat dry weight (g)	Total no. *B. fusiformis* seeds retrieved	Mean no. seeds per scat (range)	No. seeds of *B. fusiformis* per g of scat
Quokka	16	1.076	19.48	66	4.13 (0-29)	3.4
Gilbert's potoroo	141	0.361	41.01	18	0.13 (0-4)	0.4
Bush rat	61	0.038	2.61	79	1.29 (0-20)	30.3
Quenda	57	0.210	19.10	0	0	0

Table 2. Percentage germination of Billardiera fusiformis *seeds retrieved from faecal samples of three mammals compared to seeds collected directly from plants (control). Seeds from control and scats were tested fresh and aged; and aged seeds were tested with and without smoke treatment. Reproduced from Cochrane* et al. *(2005).*

	Control (seed from plant)	Gilbert's potoroo	Quokka	Bush rat
Fresh (no treatment)	0	31 ± 9.2	58 ± 1.4	2 ± 11.3
Fresh (+ smoke)	12 ± 1.6	n/a	n/a	n/a
Aged 15+mths (no treatment)	16 ± 5.3	50 ± 12.8	49 ± 7.7	32 ± 4.2
Aged 15+mths (+ smoke)	91 ± 3.4	62 ± 5.0	n/a	64 ± 4.4

will recruit successfully at a given time and in a given place. Early seedling emergence in unpredictable environments may assist in maximising seedling survival.

Plants cannot move across the landscape unaided and endozoochory may confer ecological benefits by promoting substantial and dispersed seedling recruitment. Seeds dispersed in faeces have a ready supply of nutrients and should have a competitive advantage for germination in nutrient-poor sites. Like seeds adapted to survive in the soil, those adapted to survive ingestion are small, round and hard (Pakeman *et. al.* 2002). Seeds that can build up in the soil seedbank can have a significant effect on species richness and abundance after disturbance events. In the colonisation of new or extremely disturbed sites, vegetation dynamics may be driven by immigrant seeds where a pre-existing soil seedbank is absent or depauperate. And in fire-driven environments, when fire is absent for long periods of time, endozoochory may be an important contributor to successful seed germination and establishment of plant species.

When plants depend on animals for seed transport they are susceptible to dispersal failure if their seed vectors become rare or extinct (Willson 1992). Where natural regeneration is dependent on seed dissemination, failure to disperse propagules could deplete plant populations. Low seedling recruitment and even local extinction of populations may occur. Small mammal species have experienced declines or complete extinction in many parts of Western Australia due to the introduction of foxes and cats, land clearing and changed fire regimes. Their demise may therefore contribute to changes in the balance of vegetation communities.

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FloraBank lives again

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In the last issue of *Australasian Plant Conservation*, an article by Ben Cavuoto from the Capital Region office of Greening Australia briefly described the development of FloraBank, the native seed information and web tool resource. Funded by the Australian Government through the Natural Heritage Trust, FloraBank aims to improve the availability and quality of native seed for revegetation and conservation purposes. FloraBank has been around since the late 1990's, when it first began publishing its 10 FloraBank Guidelines, Model Code of Practice and Fact Sheets. And even though the original funding for FloraBank ran out in 2001, the website remained live and as popular as ever, logging an average of 3000 hits per month during its 'dormant' period. The Australian Government recognised that the need for a high-quality, native seed information resource still exists, and the new FloraBank is now up and running, managed by Greening Australia.

Leaders in seed attend workshop on seed production areas

The new FloraBank website will maintain many of its popular and useful tools, such as the original 10 Guidelines. Over the next two years, FloraBank will be updating and revising many of the Guidelines. The first on the list is Guideline 7: Seed Production Areas for Woody Native Plants. As part of this effort, *Exchange:*

the National Vegetation Knowledge Service recently hosted a two-day workshop in Canberra to discuss how seed production areas can help meet the unmet demand for native seed in revegetation projects in Australia. The workshop was attended by some of Australia's leading seed scientists, plant geneticists, seed suppliers, and natural resource managers.

One objective that was reinforced at the workshop is that FloraBank will work with regional organisations to develop seed supply strategies. Regional targets of tens of thousands of hectares of revegetation are at risk because no one is making sure we have enough seed. Without seed supply strategies, regional plans may be likened to plans for building supermarkets, but having no food to sell. Currently, Catchment Management Authorities are required to develop Catchment Action Plans, which often include detailed and ambitious revegetation targets. While these revegetation targets are admirable and necessary for the conservation of Australia's unique vegetation, clear strategies to meet the seed requirements for these targets – which can be in the order of many tonnes of seed – are often not part of the Plan. This lack of appropriate native seed may put the significant investment in natural resource management at risk. Seed production areas, if appropriately designed and managed to meet local and regional demand, will be one solution.

Nationally accredited training workshops coming in the next year

Another way to ensure the quality of Australian native seed is through the development and provision of training workshops that set nationally recognised, common benchmarks for seed collection and supply. FloraBank is in the process of developing workshops on seed harvesting and technology for each state and territory. The workshops will draw on the expertise of Greening Australia and ENSIS (a joint enterprise of CSIRO and SCION – a New Zealand forest research group) as well as experts in the seed industry. The training will be accredited against National Competency Standards by Greening Australia's training arm, to ensure a consistently high level of training is delivered.

Figure 1. Ben Cavuoto from Greening Australia Capital Region shows native seed collected at a recent workshop. Photo: Greening Australia Capital Region.

New tools from FloraBank and an opportunity for research scientists

The FloraBank website is also being further developed to ensure that locally and regionally adaptable online decision support tools for the native seed industry are widely available. Initially links have been made to Greening Australia's Native Vegetation Research and Resource Guides, with up-to-date information on native seed research, best management practices, and key references. The Guides are contextual manuals for finding and using resources on native vegetation, drawing from over 1050 sources of research, reports, and practical information. The Guides are free of charge and are available from Greening Australia in either paper or electronic forms.

Also in the pipeline for FloraBank are the following tools:

- Species Selector Key, which uses LUCID software to allow natural resource management practitioners in pilot regions to select appropriate species for their site and purpose;
- Provenance Selector Key, which enables the development of species provenance ranges for revegetation sites where local provenance is important (i.e. seed needs to be sourced from the local area); and
- Vegetation Management Selector, which helps natural resource management practitioners to determine the best revegetation option for their site or project objectives.

Both the Species and Provenance Selector Keys will provide an easy way for people to get species-level information about plants used in revegetation, such as how many plants to collect from, minimum population size, adaptability to different environments, germinability and collection times. For plant scientists, the keys will provide a way for new science to quickly reach people who can use it. For example, research on the pollination vectors of a particular species can be used in the design of seed production areas, or to decide how widely to collect seed in order to maximise genetic diversity. Researchers interested in contributing to the key should contact the FloraBank team.

FloraBank hopes to make a positive impact on both the quality and quantity of Australian native seed for conservation. With better information, seed suppliers will be able to offer greater quantities of seed of more species, from more populations and with higher quality.

The result for seed buyers will be more revegetation, with greater species numbers and genetic diversity. Plants established from these seeds will be healthier, more likely to survive to reproduce and will produce seeds with adequate genetic diversity to adapt to a changing environment.

For more information, please log on to the FloraBank website at www.florabank.org.au.

Figure 2. Seed Production Area in the Murray Catchment. Photo: Murray CMA

The Auckland Botanic Gardens Threatened Native Plant Garden

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The Threatened Native Plant Garden has proved to be one of Auckland Botanic Gardens foremost attractions since its formal opening in 2001 by the Rt Hon. Helen Clark, Prime Minister of New Zealand/Aotearoa. Accolades have been showered upon the garden from wide-ranging audiences and visitors have cherished the opportunity to become informed about our unique and treasured natural heritage.

The Threatened Native Plant Garden is probably unique in New Zealand in that threatened plants have been arranged ecologically, together with associated non-threatened taxa. The presentation of replicated habitats has considerably more value, meaning and interest and provides further interpretative opportunities as opposed to just a collection of labelled threatened plants.

Objectives of the Threatened Native Plant Garden

The broad objectives of the garden are to create an awareness of Auckland's threatened plants and to highlight the reasons why they are in decline and what can be done to reverse their decline. Other objectives are to: assist in threatened plant identification; increase and disseminate knowledge of threatened plant propagation and cultivation; and provide plant material for research, displays and cultivation, thereby reducing pressure on the wild populations.

It was decided from the onset that the garden would have an emphasis on regionally and nationally threatened plants occurring naturally in the Auckland Region, and plants from northern offshore islands. This reflects the Garden's geographic location and plant conservation policy. Replicating habitats, albeit a mere 'snapshot' of our wild environment, has meant that we have been able to show the natural diversity of the Auckland region from the biodiverse forest-clad Waitakere Ranges in the west to the local lava-fields of what is now the industrial suburb Penrose!

Replicated habitats within the Threatened Native Plant Garden

The gardens include replicated habitat from coastal and inland environments and also from off-shore islands which provide an important refuge for many threatened plants.

Inland habitats

Inland habitats featured in the garden are diverse and include inland forest and scrub, lava-fields, gumlands, freshwater swamps and rocky bluffs.

The **inland forest and scrub** was once the most common vegetation type in New Zealand. This garden showcases colonizing species such as Manuka (*Leptospermum scoparium*), Kanuka (*Kunzea ericoides*), and canopy species such as Kauri (*Agathis australis*).

The garden also replicates the **inland rocky bluffs** which occur in the Waitakere Ranges, just north of Auckland City. The naturally restricted Rock Koromiko (*Hebe bishopiana*) which grows on rocky, moist seepages is a feature of this garden.

Coastal bluff is one of the replicated habitats featured in the Threatened Native Plant Garden. Photo: Jack Hobbs

A **wetland** garden features the rare sedge *Carex subdola.* Over 90% of New Zealand's wetlands have been destroyed and they are among some of New Zealand's rarest and most at-risk ecosystems.

One of the more unique replicated habitats is the **lava-field**. Once quite common in the Auckland region, now only small remnants of lava field remain, except on Rangitoto Island. The garden features Necklace fern (*Asplenium flabellifolium*) and Titoki (*Alectryon excelsus*).

Coastal habitats

Replicated coastal habitats include coastal forest and scrub, salt marsh, sand dunes, shell banks, boulder beach and a coastal bluff. Funding for many of the coastal habitats was secured earlier this year and works have only recently commenced. Landscaping has been completed and planting has commenced. The local Puhinui Reserve on the Manukau Harbour has regionally significant saline wetlands and provided the ecological data for the replication of coastal habitats.

A **salt marsh** habitat will include a replicated sequence of vegetation zones, including below- and mid-tide, and lower-, middle-, and upper-marsh. Threatened species will include New Zealand Spinach (*Tetragonia tetragonioides*), *Mimulus repens* and *Puccinellia stricta.* Pristine examples of salt marsh are now scarce within the region due to coastal developments.

A **sand dune** habitat will include a fore-dune, stabilised dunes and a dune forest. The replicated habitat will interpret the fragility of coastal dunes and explain the importance of their integrity in maintaining a diverse coastal ecology. Coastal dune systems within the Auckland region are collapsing due to inappropriate recreational disturbance. Interpretation will include solutions on how everyone can help protect these fragile ecosystems. A replicated mobile fore-dune will feature the endemic sand-binding plant pingao (*Desmoschoenus spiralis*) and a stabilised dune system will be established showing the transition from mobile dune to dune forest and will feature the regionally threatened *Hebe diosmifolia* and *Pseudopanax ferox.*

The construction of a replicated saline wetland habitat. Photo: Jack Hobbs

A **shell bank** habitat will feature transient species such as New Zealand Spinach, now rarely found in the region, and the closely related, commonly occurring native spinach (*Tetragonia implexicoma*). The threatened Sand Tussock (*Austrofestuca littoralis)* and Cook's Scurvy Grass (*Lepidium oleraceum*) will also feature.

The **coastal bluff** habitat features the critically endangered Napuka (*Hebe speciosa*); nationally rare and locally presumed extinct *Leptinella rotundata*, a gynodioecious plant threatened by coastal erosion, weed invasion and low seed set due to having bisexual flowers on some plants and single-sex flowers on others; *Sonchus kirkii*, the native Puha which was thought to be extinct until a few years ago when it was rediscovered on Auckland's West Coast; and *Scandia rosifolia*, *Poa anceps*, *Polystichum neozelandicum* and many more.

We look forward to welcoming our Australian visitors to this dynamic and educational resource.

Report from New Zealand Plant Conservation Network

Bec Stanley

Email: rebecca.stanley@arc.govt.nz

In June this year our website passed through 10 million hits making it the most visited plant information site in the country. Several new features continue to keep the site active and interesting, including an animal pest database to highlight the devastating effects introduced mammals have had on our flora (and fauna), a moss and liverwort database, and enhanced search capability to find threatened plants and fungi by location. Another popular new feature is the ability to email plant Fact Sheets to others by the click of a button. The aim of the plant Fact Sheets is to complete them for all of the New Zealand Threatened Flora in the medium term but ultimately the whole vascular flora. To date Fact Sheets for all the Acutely Threatened Vascular Plant Taxa have been completed and updated.

Fact sheet for the extinct Trilepidea adamsii *from the New Zealand Plant Conservation Network website <http://www.nzpcn.org.nz/>*

NZPCN is now working on the much larger grouping of 'At Risk' taxa. New Zealand's threatened plants are listed by an expert committee using a threat classification system developed by the New Zealand Department of Conservation (DOC) (Molloy *et. al.* 2002). The result of this process is a list of 797 native vascular plant species (de Lange *et. al.* 2004) that can be downloaded directly from the NZPCN web site.

The plant checklist section of the newsletter is proving a valuable resource with lists by local botanists posted regularly on the website. They are used by local government ecologists, Department of Conservation botanists, landscape architects, and students as well as by other local botanists keen to add to the knowledge of particular areas.

Our annual web-based poll to discover 'New Zealand's favourite plant' has been launched and with over 30,000 visitors a month to the website we expect strong interest and are eagerly awaiting the results in early December. The aim of the poll is to inspire interest in our native flora and to raise awareness of the website. Results will be announced at the Network conference in November in Auckland that celebrates the centenary of the publication of the first full flora to be published by a resident New Zealand botanist, Thomas F. Cheeseman's *Manual of the New Zealand Flora* (1906).

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de Lange, P.J., Norton, D.A., Heenan, P.B., Courtney, S.P., Molloy, B.P.J., Ogle, C.C., Rance, B.D., Johnson, P.N. and Hitchmough, R. (2004). Threatened and uncommon plants of New Zealand. *New Zealand Journal of Botany* 42: 45-76.

Molloy, J., Bell, B., Clout, M., de Lange, P., Gibbs, G., Given, D., Norton, D., Smith, N. and Stephens, T. (2002). Classifying species according to threat of extinction - a system for New Zealand. Department of Conservation, Wellington.

View *Trilepidea*, the E-Newsletter of the New Zealand Plant Conservation Network:

August 2006
http://www.nzpcn.org.nz/documents/Trilepidea-060822.pdf

September 2006
http://www.nzpcn.org.nz/documents/Trilepidea-060911.pdf

Book Review

Flora of the Otway Plain & Ranges. 1. Orchids, Irises, Lilies, Grass-trees, Mat-rushes and other petaloid monocotyledons

By Enid Mayfield

Linton Press, Geelong, 2006, 219 pages, numerous colour illustrations, paperback.

ISBN 0 977571203. Price $45.00.

To order contact lintonpress@westnet.com.au

Despite the title, this book isn't just about the Otways or monocotyledons; it offers much more than most other field guides and deserves a place on the bookshelves of everyone interested in natural history. I couldn't think of a better text for the beginner, but I'm confident that practising botanists would learn new things from this book – and it will prove to be an excellent teaching tool. The author has obviously spent time planning every meticulous detail, and it shows. The contents are interesting and accessible and every section is accompanied by the most exquisite, clear and unambiguous colour illustrations which will instantly captivate the reader.

Petaloid Monocotyledons are its specific subject; but the book contains a large amount of associated information; indeed everything of interest and relevance seems to have been included. Characteristics of each plant family are explained and there are easy visual guides to recognition of flowers from major groups and illustrated keys to species. Latin names are given meaning and in many instances historical detail is also included. The general content includes maps, an illustrated glossary, a comprehensive index and a bibliography.

The descriptions are presented in alphabetical order of families, genera then species. Colour illustrations for each taxon accompany precise information necessary for identification. Under each species are included common names, brief recognition guides, the geographical occurrence (including for the rest of the continent and further afield), and habitat, status and flowering times. The section on orchids is particularly fascinating. Symbiotic and exploitative relationships play an integral role in healthy ecosystem functioning, but I've not seen them treated so well in other field guides. Insects are crucial to orchid pollination, but here all is explained and illustrated brilliantly. An example is the extraordinary orchid *Thynninorchis huntianus* [see cover illustration], where its partial dependence on a mycorrhizal fungus for nutrition and its exploitation of a flower wasp for pollination are revealed.

The design is well thought out and very attractive; most pages are partitioned into boxes or blocked colour in order to highlight important details but this is done in a

restrained manner which doesn't dominate the text or illustrations. The illustrations are extremely elegant, revealing the intricate detail needed for accurate identification and demonstrate the importance of botanical illustration in understanding the construction and workings of organisms.

First impressions can be important, and mine was that this is a quality publication. The book's cover is attractive; the cover is reasonably sturdy, but I'll cover mine with protective plastic because I know I'll be using it often – even though I live a long way from the Otways! The size and shape are good and the pages are silky and smooth to handle. It's well-made, too, sewn in sections which won't fall apart, and, except for the extreme ends, the book stays open at the pages in use.

Why should every natural historian buy this book? It has enough to satisfy every enthusiast, and – this is what I find really impressive – it is so easy to use that it will encourage further investigation of its subjects. The author summarises it well in the last line of her introduction: "This is the book I wanted to have when I went out into the field."

Katrina Syme, Denmark, Western Australia

Research Roundup

Batty, A.L., Brundrett, M.C., Dixon, K.W. and Sivasithamparam, K. (2006). ***In situ* seed germination and propagation of terrestrial orchid seedlings for establishment at field sites**. *Australian Journal of Botany* 54(4): 375-381.

Cabeza, M. and Moilanen, A. (2006). **Replacement cost: a practical measure of site value for cost-effective reserve planning**. *Biological Conservation* 132(3): 336-342.

Caley, P. and Kuhnert, P.M. (2006). **Application and evaluation of classification trees for screening unwanted plants**. *Austral Ecology* 31(5): 647-655.

Callaway, R.M. and Maron, J.L. (2006). **What have exotic plant invasions taught us over the past 20 years?** *Trends in Ecology and Evolution* 21(7): 369-374.

Cumming, G.S. and Spiesman, B.J. (2006). **Regional problems need integrated solutions: pest management and conservation biology in agroecosystems**. *Biological Conservation* 131(4): 533-543.

Dorrough, J., Moxham, C., Turner, V. and Sutter, G. (2006). **Soil phosphorus and tree cover modify the effects of livestock grazing on plant species richness in Australian grass woodland.** *Biological Conservation* 130(3): 394-405.

Hastings, R.A. and Beattie, A.J. (2006). **Stop the bullying in the corridors: can including shrubs make your revegetation more Noisy Miner free?** *Ecological Management & Restoration* 7(2): 105-112.

Liddle, D.T., Brook, B.W., Matthews, J., Taylor, S.M. and Caley, P. (2006). **Threat and response: a decade of decline in a regionally endangered rainforest palm affected by fire and introduced animals**. *Biological Conservation* 132(3): 362-375.

Lindenmayer, D.B., Franklin, J.F. and Fischer, J. (2006). **General management principles and a checklist of strategies to guide forest biodiversity conservation.** *Biological Conservation* 131(3): 433-445.

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Scade, A., Brundrett, M.C., Batty, A.L., Dixon, K.W. and Sivasithamparam, K. (2006). **Survival of transplanted terrestrial orchid seedlings in urban bushland habitats with high or low weed cover.** *Australian Journal of Botany* 54(4): 383-389.

Scott, K. (2006). **Effect of heat on the dormancy and viability of *Parkinsonia* seeds: implications for management**. *Ecological Management & Restoration* 7(2): 153-163.

Tasker, E.M. and Bradstock, R.A. (2006). **Influence of cattle grazing practices on forest understorey structure in north-eastern New South Wales**. *Austral Ecology* 31(4): 490-502.

Thomas, J., Hofmeyer, D. and Benwell, A.S. (2006). **Bitou Bush control (after fire) in Bundjalung National Park on the New South Wales North Coast**. *Ecological Management & Restoration* 7(2): 79-92.

Williams, P.R. (2006). **Determining the management requirements of threatened plant species**. *Ecological Management & Restoration* 7(2): 148-153.

Information Resources and Useful Websites

Introduction to plant and fungal interactions

University of Sydney Mycology Course Website

http://bugs.bio.usyd.edu.au/Mycology/Plant_Interactions/plantInteractions.shtml

Information on mycorrhizas, endophytes and other plant-fungus interactions, with details and illustrations of the different types of mycorrhizas, such as ectomycorrhizas.

Soil biodiversity the key to sustainable agriculture?

Improved crop yields are being enjoyed by some Mexican farmers with the help of soil bacteria and fungi rather than artificial fertilizers. These results are part of a United Nations Environment Programme (UNEP) project aimed at understanding and harnessing 'below ground biodiversity' for sustaining, restoring and improving the fertility of the land. Three years into the project, the Mexican researchers are also unearthing new species including three new species of ants and up to 15 new species of mycorrhizal fungi – organisms that help the roots of plants extract minerals and water from the soil.

Read more at: http://www.unep.org/Documents.Multilingual/Default.asp?DocumentID=471&ArticleID=5236&l=en.

International Year of Deserts and Desertification Factsheets

http://www.deh.gov.au/events/iydd/factsheets.html

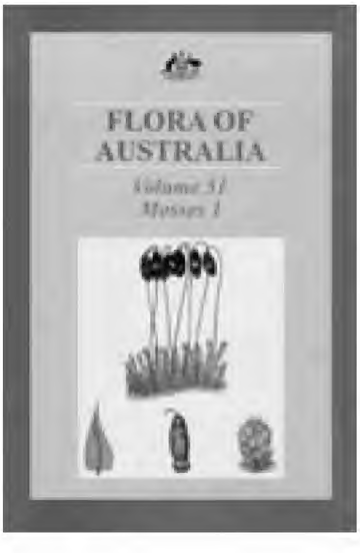
These factsheets have been produced by the Australian Government Department of the Environment and Heritage as part of activities around the International Year of Deserts and Desertification. Topics are: Deserts in Australia, Desert fauna, Desert flora, Desertification – a global issue, and The desert people of Uluru-Kata Tjuta National Park.

Flora of Australia. Mosses

Flora of Australia. Volume 51.

P.M. McCarthy (Ed.), 2006, ABRS & CSIRO Publishing

The first of three volumes describing and illustrating more than 1,000 species of Australian mosses. Includes an introduction documenting research on Australian mosses, and sections on moss classification, ecology and biodiversity, fossil bryophytes and the origin and evolution of mosses. This volume covers mosses from the family Sphagnaceae to Hypopterygiaceae. ISBN 0643092404.



Supplement to Native trees and shrubs of south-eastern Australia

Leon Costermans, 2006, Costermans Publishing, Frankston

The 'Costermans' has been an invaluable guide to trees and shrubs in SE Australia since its publication in 1981. This supplement provides information on about 230 new or additional species and details modifications to the information for another 250 species in the original book. CD-ROM, with high and low resolution versions for screen and also version suitable for printing. RRP $25. Order from VNPA at http://www.vnpa.org.au/resources/publications/costermans.htm

Native vegetation of the Murray region

Todd Berkinshaw, 2006, Mid Murray Local Action Planning Committee, Cambrai

A comprehensive guide to the identification, protection and restoration of native vegetation communities and plant species of the South Australian Murray Darling Basin, with photographs. Accompanied by an interactive CD-ROM version. ISBN 0977514307.

Cooperative Orchid Conservation

http://www.rbg.vic.gov.au/coc/home

The Cooperative Orchid Conservation website has been developed by a number of organisations actively involved in orchid conservation including the Royal Botanic Gardens Melbourne, the Department of Sustainability and Environment, the Royal Melbourne Zoological Gardens, the Australasian Native Orchid Society, the University of Melbourne and RMIT University.

The purpose of the website is to inform interested people about the range of orchid conservation work that is happening in Australia, and to help people that are seriously involved in orchid conservation to link up with others that are working to improve the conservation status of Australia's threatened orchids.

CSIRO Sustainability Network

http://www.bml.csiro.au/sustnet.htm

The CSIRO Sustainability Network is a 'virtual learning community of practice' dedicated to inclusive exchange of ideas and information on sustainability and sustainable development – across science disciplines within CSIRO, and across professions, disciplines and generations generally. The several hundred listed members include CSIRO scientists, but the majority are in business, industry, education, government, NGOs, and the community.

Informal newsletters are produced every 3-4 weeks. Topics covered in the latest newsletter (No. 61) include: Soil fertility management for more sustainable farming systems; Sustainable agriculture and the challenge to make it pay; Downsizing product packaging; Eco burial as an environmental donation; Jet travel as a 'sin'; and feedback on Nuclear energy.

ANPC Workshops

'From the Ground Up'
Workshop on conservation and rehabilitation of grassy ecosystems of the ACT and region

28-29 November 2006

CSIRO Discovery Theatre, Clunies Ross Street, Acton, ACT.

Workshop structure and content

This two-day workshop will cover topics such as: identifying grassy ecosystems (treeless grasslands, grassy woodlands), ecological principles underlying successful rehabilitation, the landscape context of a site, rehabilitation planning, assessment of site and vegetation condition, soil health and the role of soil-symbionts, management of threats, monitoring progress and ongoing management, learning from case studies.

We will visit two field sites differing in condition and management. Participants will break into smaller groups for demonstrations and trialling of techniques, discussion of management issues and rehabilitation approaches and some field-based plant identification.

Who should come?

Anybody interested in or responsible for the conservation, management or rehabilitation of grassy ecosystems in the ACT and local region. This includes community groups (coordinators and volunteers), extension and support officers, government agency staff, local government, environmental consultants, landholders and other land managers, other decision-makers and anyone interested.

Why 'from the ground up'?

The title reflects some important aspects of the workshop:

- thinking rehabilitation from the basics (an ecological approach);
- including soil health and soil-plant associations in rehabilitation planning;
- supporting grass-roots community workers and volunteers; and
- improving expertise of all working in on-ground conservation management.

Registration

For information on registration fees, see the registration box below.

Project partner: Environment ACT.

Project supporters: Greening Australia (ACT & SE NSW), Friends of Grasslands (FOG), Ginninderra Catchment Group, Southern ACT Catchment Group, Molonglo Catchment Group, Monaro Grasslands Conservation Management Network.

This project is supported by an ACT Environment Grant.

Registration information for ANPC workshops

Registration forms, flyers and programs are available on the ANPC website http://www.anpc.asn.au/course1.html or from the ANPC Office.

The registration fee covers catering (morning and afternoon tea, and lunch), field trips, the ANPC Plant Conservation Techniques manual on CD and includes GST. This fee covers costs not met by grants.

ANPC member: $175 *Concession: $85

Non-member: $195 *Concession: $105

* Concession for non-employed volunteer community group members, full-time students, pensioners.

Accreditation: Participation in ANPC workshops can contribute to qualifications in the Conservation and Land Management Training Package. Cost = $25 (incl. GST). More information available on request and on ANPC website: http://www.anpc.asn.au/course1.html#Courseaccreditation.

ANPC Workshops (continued)

Workshop on rehabilitation and management of disturbed native vegetation

Coffs Harbour, NSW 14-15 March 2007

Are you involved in the rehabilitation of native vegetation? Does your work-site include native vegetation that you need to manage or rehabilitate? Do you participate in local rehabilitation projects? Are you interested in reversing the decline of native plant communities in your area? Then this workshop is for you!

Workshop focus

- The knowledge and skills required to undertake ecological rehabilitation and management of disturbed native vegetation.

Themes include

- The ecological principles essential to successful rehabilitation and management;
- Understanding the task (the goal for the site, planning, resources, assessing site condition and resilience, issues such as soil health, provenance, urban impacts, monitoring and ongoing management, local issues); and
- Applying ecological principles to rehabilitation (case studies, site visits, demonstration and trialing of techniques).

Registration

For information on registration, see the registration box on p. 29.

This workshop is subsidised by the NSW Government through its Environmental Trust.

ANPC National Forum – April 2007
What lies beneath? The role of soil biota in the health and rehabilitation of native vegetation.

17-19 April 2007

CSIRO Discovery Theatre, Black Mountain, Acton, Canberra, ACT.

Forum Theme

This national three-day forum will provide an opportunity for the sharing of the latest research outcomes on the role of soil organisms in ecosystem function and native vegetation rehabilitation. Practitioners will also demonstrate application to rehabilitation practice, share knowledge and skills gained from experience, and identify areas of research needed to fill knowledge gaps.

The forum theme is a response to a demand for scientific and practical guidance on this often overlooked but fundamental aspect of natural resource management.

The forum will include:

- presentations on the role of soil biota in ecosystem function and native vegetation rehabilitation
- workshops, panels and discussions
- field visits demonstrating techniques and practical application.

Further information will be posted on the ANPC website as the forum evolves.
http://www.anpc.asn.au/conferences.html

Workshop on the Translocation of Threatened Plants – to be confirmed

20 April 2007 (immediately following the national forum)

CSIRO Discovery Theatre, Clunies Ross Street, Acton, ACT.

This workshop is essential for anyone involved in the planning, approval or implementation of translocation projects for threatened flora across Australia. The workshop is particularly relevant for environmental consultants and local government staff involved in the development approval process. The presenters include translocation experts who were involved in developing the *Guidelines for the Translocation of Threatened Plants in Australia* (ANPC, revised edition 2004) as well as selected case studies, highlighting lessons to be learnt.

Registration not open until workshop confirmed.

For further information on any of the above, contact:

ANPC National Office
Phone: 02 6250 9509 or email: anpc@anpc.asn.au

Conferences and Workshops

Benefits of Native Vegetation in Agriculture Training Series

October 2006 - February 2007

Five day training series for farmers and land managers who want to raise farm production and income while increasing long term viability of natural farm resources, ecology and aesthetics. Sessions include Farm Forestry and Native Vegetation Use in the Management of Water Quality. Location: The Bridge Community House (Shire of Yarra Ranges) Kilsyth, Victoria. Oct. 6, Nov. 10, Dec. 8, 2006, Jan. 19 & Feb. 16, 2007. Free event organised by Greening Australia. Bookings are essential as places are limited.

Further information:
Rebecca Passlow, RPasslow@gavic.org.au.

Rick Farley Lectures 2006

Sponsored by the NSW Natural Resources Advisory Council, October - November 2006

The theme for this series of evening lectures is 'Respect and care for the land and waters we share'. Six different lectures will be held in Sydney, Armidale, Coffs Harbour, Bathurst, Wagga Wagga and Newcastle, each presented by prominent speakers: Mike Archer, Larissa Behrendt, Ian Lowe, Wendy McCarthy, Phillip Toyne and Robyn Williams. Lectures are free, but tickets are required.

Further information:
http://www.nrac.nsw.gov.au/farley/intro.html

Plant Diversity in the Tropics Australian Systematic Botany Society Conference

13-15 November 2006, James Cook University, Cairns

Plant Diversity in the Tropics will explore a variety of themes including the growing link between modern plant systematics and the conservation of rare and threatened species. 2006 is also the 400th anniversary of first Dutch contact with Australia. The conference will explore the Dutch contribution to Australasian systematic botany. A workshop and masterclass 'Molecular tools in plant systematics' will be held on the day following the conference.

See: http://www.anbg.gov.au/asbs/conferences/

New Zealand Plant Conservation Network Conference

20-22 November 2006 (including field trip), Conference Centre, University of Auckland

This year's Network conference will be the Cheeseman Symposium 2006 – to celebrate the centenary of the publication of the first full flora treatment to be published by a resident New Zealand botanist, Thomas F. Cheeseman's *Manual of the New Zealand Flora* (1906). The conference will be held in conjunction with the New Zealand Botanical Society, Auckland Museum, Auckland Botanical Society, Landcare Research and the University of Auckland.

See www.nzpcn.org.nz (under Conservation info> Events>Conference) for details and registration.

Seed Workshops in Victoria

October 2006 to February 2007

Introduction to seed collection is an informal introduction to all facets of seed collection. Sessions will be held at:

- Ballarat, Apollo Bay, Geelong, Inverleigh and at locations in the North Central Region of Victoria, Oct. to Dec. 2006. Contact: Anne Ovington, anne.ovington@dpi.vic.gov.au
- South Gippsland, Dec. 2006. Contact Drew Liepa, DrewL@wgcma.vic.gov.au
- Yarram region, Mullungdung Flora and Fauna Reserve, 20 Nov. 2006. Contact: Martin Potts, mpotts.gav@dcsi.net.au

Seeds to Success is a five day accredited course designed for people with an interest in collecting seed from native vegetation, including data collection, extraction, cleaning, storage and propagation. The courses are at the Victorian Landcare Centre, Creswick, Nov.-Dec. 2006 and Jan.-Feb. 2007. Contact: Anne Ovington, anne.ovington@dpi.vic.gov.au

Conferences and Workshops (continued)

Southern Connection Congress

22-26 January 2007, University of Adelaide, South Australia

Southern Connection is a group of scientists from all continents who study aspects of biology and earth history of the southern continents. The conference has an arid-zone theme, plus a field trip to Kangaroo Island.

Symposia include:

- Aridification on the four southern continents: Australia, South America, Africa and Antarctica. Timing of desertification and implications for biota;
- Southern temperate marine ecosystems;
- Goodbye Gondwana: a fresh perspective on the roles of vicariance and dispersal in southern hemisphere biogeography;
- Methodologies for studying southern urban ecosystems; and
- Understanding the impact of invasive species.

See: http://events.lincoln.ac.nz/southern/events.htm, or email Glenn Stewart stewartg@lincoln.ac.nz

3rd Global Botanic Gardens Congress 'Building a Sustainable Future: the Role of Botanic Gardens'

16-20 April 2007, Wuhan, China

The Global Botanic Gardens Conference is held every three years. The 2007 Conference marks the 20th anniversary of Botanic Gardens Conservation International (BGCI) and will provide a global forum for the botanic garden community to share their knowledge, experience, practice and research.

Further information: http://www.3gbgc.com

Conserv-vision, the next 50 years: an international conference on conservation of biodiversity and historic resources

4-7 July 2007, University of Waikato, Hamilton, New Zealand

This conference celebrates the first 20 years of New Zealand's Department of Conservation.

See: http://www.waikato.ac.nz/wfass/Conserv-Vision/

Australasian Section of the Society for Conservation Biology

10-13 July 2007, University of New South Wales, Sydney

An inaugural Regional Meeting of Conservation Scientists, organised by the Australasian Section of the Society for Conservation Biology, will be held next year on the topic 'The Biodiversity Extinction Crisis, a Pacific and Australasian response'.

Special challenges in the Pacific and Australasian region include: island ecology, rising sea levels, changing rainfall, and land and water degradation. These issues are overlaid by the general problems of habitat loss and fragmentation, invasive species, pollution and over-harvesting. The conference will identify major problems for biodiversity conservation in the region, look for existing and potential solutions and establish links to global biodiversity initiatives.

There will be five major themes:

- Regional challenges (particular issues for our part of the world);
- Managing threatening processes of universal importance;
- Case studies of conservation in action, including biodiversity monitoring and assessment;
- Conservation science and policy; and
- Conservation science and the community (NGOs, indigenous people).

9th International Conference on the Ecology and Management of Alien Plant Invasions

17-21 September 2007, Hyatt Regency Hotel, Perth, Western Australia

Further information: http://www.congresswest.com.au/emapi9

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Coffs Harbour City Council, New South Wales

Department for Environment & Heritage, Science and Conservation, South Australia

Department of Environment & Conservation, New South Wales

Department of Environment and Conservation, Western Australia

Department of Natural Resources, Environment and The Arts (NRETA), Northern Territory

Department of Sustainability and Environment, Warrnambool, Victoria

Ensis Genetics, Australian Tree Seed Centre, ACT

Redland Shire Council, Queensland

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Parks, Conservation and Lands Environment and Recreation

Australian Network for Plant Conservation, Inc
presents

'From the Ground Up': a workshop on the conservation and rehabilitation of grassy ecosystems of the ACT & region

Canberra Tuesday 28 - Wednesday 29 November 2006

Venue: CSIRO Discovery Theatre, Clunies Ross Street, Acton

Are you interested in native grasslands? Are you involved in managing and rehabilitating grassy ecosystems? Do you participate in local rehabilitation projects? Are you interested in reversing the decline of these fascinating plant communities? Then join us for this workshop!

Workshop focus:

Improving knowledge and skills for ecological conservation management of grassy ecosystems.

Themes include:

- Ecological principles essential to successful management and rehabilitation;
- Understanding the task (the goal for the site, planning, assessing site and vegetation condition, the landscape context of the site, issues such as soil health and the role of soil-symbionts, provenance, management options, threats such as weeds, monitoring progress and ongoing management);
- Applying ecological principles (local case studies, demonstration and trialling of techniques).

The workshop will be a mix of presentations by grassy ecosystem specialists and visits to selected field sites. Participants will extend their skills and knowledge, exchange ideas and expertise, work with experts and broaden networks.

Why 'from the ground up'?

- rehabilitation from the basics (ecological approach)
- soil health & soil-plant associations
- assisting grass-roots community volunteers
- improving skills for on-ground conservation management

NSW Environmental TRUST

Workshop fee (includes catering, field trip, Plant Conservation Techniques manual on CD & GST):

ANPC member: $175 *Concession: $85
Non-member: $195 *Concession: $105

* Concession for volunteer community group members, full-time students, pensioners.

Registration forms available from the ANPC (website or office - details below)

Registrations close: Friday 17 November 2006

Accreditation: Participation in ANPC workshops can contribute to qualifications in the Conservation and Land Management Training Package. Cost = $25 (including GST). More information available on request (and on ANPC website).

Photographs (top to bottom): *Themeda triandra* in flower; photo by Geoff Robertson. FOG members investigating the grassland in Scabby Nature Reserve, Yaouk; photo by Geoff Robertson. Greenhood orchid; photo by Geoff Robertson. Monaro grassland on basalt (Poa Tussock and Kangaroo Grass with Blue Devils flowering in the foreground), Ravensworth TSR near Cooma; photo by David Eddy. Copperwire daisy (*Podolepis* sp); photo by Geoff Robertson.

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